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Effects of maturation, polyploidy, and nutrition on growth, composition, and gene expression within fatty acid metabolism in rainbow trout

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ABSTRACT

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Meghan L. Manor

In many cultured fish species, such as salmonids, gonadal development occurs at the expense of stored energy and nutrients, including lipids. Mobilization of intramuscular lipid during gonadal development decreases fillet quality. The aquaculture industry induces triploidy to generate sterile individuals; however, differences in lipid metabolism of female diploid (2N) and triploid (3N) trout may alter fillet quality. In addition, there is concern that genetic selection for increased growth negatively impacts fillet quality and muscle lipid content. Research in these areas would aid in the development of better management practices for efficient food-fish production that optimizes product quality.

The goal of this research is to assess the specific impacts of sexual maturation, polyploidy, and nutrition on growth responses, fillet quality attributes, and fatty acid content. Additionally, changes in gene expression of 35 genes within the regulatory pathways governing fatty acid metabolism of various energy stores were investigated to elucidate mechanisms regulating nutrient repartitioning during sexual maturation. Four studies were conducted to assess these variables. In the first study, effects of feeding level and polyploidy on fatty acid composition and metabolism of energy stores were considered. This study showed that ploidy had greater impact on fatty acid metabolism and composition of energy stores than moderately restricted diets at sexual maturation. A second study investigated changes in fatty acid metabolism of 2N and 3N female trout throughout sexual maturation. These data showed that there are no physiological differences between 2N and 3N females prior to 18 M of age; however, there are dramatic differences in energy store compositions and gene expression beginning at 20 M of age. In general, data indicate 2N fish have increased fatty acid β-oxidation in white muscle that was associated with altered gene expression within the mTOR pathway and in visceral adipose tissue that was associated with increased *pparβ* expression. In contrast, increased expression of genes involved in fatty acid synthesis in 3N female liver appears to be associated with increased expression of PPARγ as well as altered expression within the mTOR pathway, consistent with continued deposition of lipids in these fish. A subsequent study examined differences in fatty acid composition and gene expression between immature male and female rainbow trout. Females had higher muscle polyunsaturated fatty acid (PUFA) content; albeit, no differences were observed for other fatty acids measured. Gene expression data indicate possible increased fatty acid turnover in female trout muscle through increased expression of genes involved in both fatty acid synthesis and β-oxidation. Male livers have higher expression of genes within β-oxidation, which may contribute to the lower PUFA content. Lastly, to evaluate specific associations of fillet yield and fat content with differences in fatty acid metabolism, growth, fillet fatty acid composition, and gene expression were assessed for 100 fish chosen based on fillet yields and fat contents. This study indicated that high-yield/low-fat fish produced the highest quality fillets as measured by instrumental texture and composition. In addition, high-yield/low-fat fillets had the greatest long-chain, polyunsaturated fatty acid content. Overall, data suggests that differences in growth and fillet quality phenotypes may partially result from variation in the capacity for fatty acid, β-oxidation through altered gene expression within the mTOR signaling pathway.

In general, sexual maturation, triploidy, and gender have profound affects on fatty acid composition, metabolism, and gene expression. Furthermore, the mTOR and PPAR signaling pathways have altered gene expression that is associated with differences in fatty acid composition and metabolism in rainbow trout.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The increasing world population, projected to reach over 9 billion by 2050, has resulted in a startling demand for food production. Currently, we do not produce enough food to feed that many people. Food production will have to double while reducing the environmental impact in order to achieve this feat. A changing demographic where people have greater income, allowing them to consume more protein-based diets. Additionally, the perception that fish is an excellent protein and essential fatty acid source is growing. These perceived and real issues perpetuate increased demands for aquatic foods. The captive fisheries have not been able to increase production since the 1980's (FAO, 2013). Therefore, the aquaculture industry has been forced to rapidly expand in order to supply the increased demand. The aquaculture industry is now considered the fastest growing, food-animal production sector in the world with an average annual increase of 6.1% during the last 10 years (FAO, 2013). Aquaculture accounts for roughly 50% of the world's food-fish supply and 76% of the global freshwater, finfish production (FAO, 2008). Over 77% of fish production was used for direct human consumption in 2006 (FAO, 2006). Furthermore, production has grown from under 1.0 million tons to 62.7 million tons per year over the past 50 years, and it has a value of $130.2 billion dollars worldwide (FAO, 2013). It was reported that rainbow trout alone contributed $2.2 million (FAO, 2006).

Salmonids, such as rainbow trout, are considered to be a saturated market in North and South America, as well as in Europe (FAO, 2013). However, the market is dynamic because there is an increased research interest in farmed fish that is focused on understanding fish fatty acid biochemical and metabolic pathways and improving the omega 3 fatty acid content of aquatic food products for consumer consumption (Karakatsouli, 2012). Evidence associating long-chain, omega 3 polyunsaturated fatty acids with a variety of human health benefits, seafood as the major dietary source of these fatty acids, and studies linking fish consumption to reduced cardiovascular disease have justified the continued interest in this research (Dyerberg et al., 1975; Ruxton and Derbyshire, 2009; Karakatsouli, 2012). Consequently, it is important for the aquaculture industry to produce high-quality fish, containing high amounts of omega 3 fatty acids, through appropriate production practices. Therefore, research investigating the role of fatty acid metabolism and its regulation in animal growth, reproduction, and fillet quality is required in order to maintain high standards and develop more efficient food-fish production.

There is also an increasing demand for production of polyploid salmonids (Piferrer et al., 2009). Triploid (3N) fish have three sets of chromosomes as opposed to two sets of chromosomes in normal diploid (2N) fish. The aquaculture industry induces triploidy in a variety of cultured species to cause sterility, improve growth, and prevent the onset of sexual maturation. Female 3N rainbow trout do not undergo sexual maturation and therefore, do not develop large ovaries, nor experience the shift from somatic growth to gonadal growth, thereby preventing mobilization of lipids and deterioration of muscle quality (Piferrer et al., 2009). Triploid males do, however, undergo sexual maturation, but produce non-viable sperm (Piferrer et al., 2009). These characteristics make the culture of all female, 3N fish desirable. Nevertheless, little is known about differences in 2N and 3N fatty acid metabolism and fillet quality or the regulation of these processes during maturation.

It was the goal of this research to investigate possible roles of fatty acid metabolism, and its regulation, in altering nutrient partitioning during growth and reproduction. Additional investigations into metabolic differences in 2N and 3N females and differences between male and female trout, as well as associations between gene expression and fillet quality attributes, are included in this work. There are a total of four separate projects that examine the same set of responses, including growth variables, fillet quality attributes, proximate and fatty acid compositions of energy stores, and gene expression of 35 genes within fatty acid metabolism. The first project examines the effects of ration and ploidy on these parameters at two time points during rapid gonadogenesis (Chapters 1 and 2). The second project follows changes in fatty acid metabolism throughout sexual maturation in 2N and 3N females from 16 to 24 months of age (Chapter 3). The third project looks at differences between immature male and female rainbow trout because most marketed products are from immature fish (Chapter 4). Lastly, the fourth project investigates associations between fillet quality attributes in immature female trout and gene expression profiles (Chapter 5). This research enhances our understanding of changes in fillet quality and fatty acid metabolism as affected by sexual maturation, polyploidy, gender specification, and growth. Furthermore, understanding differences in lipid utilization allows for the development of better management strategies for more efficient, food-fish production.

LITERATURE REVIEW

Fatty Acid Metabolism:

Lipoproteins: Triacylglycerol (TAG) consists of three fatty acids esterified to a glycerol backbone. TAGs are hydrophobic in nature and therefore must be transported through the circulatory system. They are transported as components of globular micelle particles that consist of a nonpolar core of TAGs and cholesterol esters surrounded by an amphiphilic coating of protein, phospholipid, and cholesterol called lipoproteins (Voet and Voet, 2004). Lipoproteins have apoproteins that are distributed on the outside of the molecules to disguise the hydrophobic contents as hydrophilic. There are nine main apoproteins that are found on human lipoproteins. They are water-soluble proteins that weakly associate with lipoproteins allowing for easy transfer among the various lipoproteins. The nine apoproteins are A-I, A-II, B-48, B-100, C-I, C-II, C-III, D, and E, and are they summarized in Table 1 (Voet and Voet, 2004).

There are five categories of lipoproteins, each with unique combinations of apoproteins, functions, and physical properties (Table 2 and Figure1). The least dense category is the chylomicrons. They are the largest lipoprotein and carry exogenous, or dietary TAGs from the intestine to other tissues. Chylomicrons consist of 90% TAGs, 4% cholesterol esters, 2% cholesterol, and 2% protein. Chylomicrons have apoproteins A-I, A-II, B-48, C-I, C-II, C-III, and E (Voet and Voet, 2004). Since chylomicrons' function is to transport dietary TAGs from the intestine to tissues, they are synthesized in intestinal cells, but their remnants are taken up by the liver (Chow, 2008). The next dense lipoprotein is very low density lipoprotein (VLDL). VLDL carries endogenous TAG and cholesterol from the liver to other tissues. VLDL consists of 65% TAGs, 15% cholesterol esters, 10% free cholesterol, and 10% protein. VLDL carries apoproteins B-100, C-I, C-II, C-III, and E, and this lipoprotein is synthesized in the liver (Voet and Voet, 2004). As VLDL circulates and loses its TAGs, it becomes smaller and denser. VLDL is then considered intermediate density lipoprotein (IDL). IDL contains 22% TAGs, 30% cholesterol esters, 8% free cholesterol, and 20% protein. IDL carries the same apoproteins as VLDL (B-100, C-I, C-II, C-III, and E) (Voet and Voet, 2004). As IDL continues to circulate and lose its TAGs, it becomes smaller and denser until it is considered the densest lipoprotein involved in TAG transport, low density lipoprotein (LDL). LDL consists primarily of cholesterol

esters (40%), and contains 10% free cholesterol, 10% TAG, and 25% protein. LDL loses most of the apoproteins, but still has apoprotein B-100 attached (Voet and Voet, 2004). The fifth lipoprotein is high density lipoprotein (HDL); its primary role is in the reverse transport of excess cholesterol from tissues back to the liver for removal through bile synthesis and excretion. HDL contains very little TAGs and therefore has no main role in TAG transport (Voet and Voet, 2004).

Dietary Fatty Acid Absorption: Dietary lipids, such as TAGs, must pass across the intestinal membrane for absorption. There are four events that must occur for TAGs to be assimilated: 1) secretion of bile and lipases, 2) emulsification, 3) enzymatic hydrolysis of ester linkages, and 4) solubilization of lipolytic products within bile salt micelles (Figure 2) (Johnson, 2001). Lipid digestion begins in the stomach; this process consists primarily of absorption of short chain fatty acids that are membrane permeable and water soluble. Cholecystokinin (CCK) is released when lipids are detected in the small intestine. CCK causes gastric motility to slow, secretion of lipases, and contraction of the gallbladder. Gastric lipases are secreted by the fundus portion of the stomach; these lipases hydrolyze TAGs to diacylglycerides (DAG) (Johnson, 2001).

In addition, several pancreatic lipases function within the intestinal lumen. Pancreatic lipase-colipase cleaves R1 and R3 ester linkages of TAGs. Phospholipase A2 cleaves R2 fatty acid and yields a free fatty acid and lysophospholipid (Voet and Voet, 2004). TAG digestion is completed by the time the bolus reaches the mid-jejunum (Johnson, 2001). Since TAGs and fatty acids are hydrophobic, they coalesce into lipid droplets with hydrophobic tails in the center and hydrophilic heads towards the outside forming a micelle. Micelle formation is important in absorption (Voet and Voet, 2004). Bile salts aid in emulsification and micelle formation. Micelles diffuse across the unstirred water layer at the intestinal lumen-membrane interface, increasing the fatty acid concentrations at the apical membrane by 100 to 1000 fold. However, medium and short chain fatty acids are not dependent on micelle formation for absorption because they are water soluble (Johnson, 2001). Once fatty acids and monoacylglycerides (MAG) are in enterocytes of the intestine, TAGs are reformed and incorporated into chylomicrons.

Although triglyceride digestion is considered to be highly conserved among species, there are marked anatomical differences in fish that have made it challenging to study metabolic and enzymatic processes (Tocher, 2005). In fish, lipolyic processes occur mainly in the proximal region of the intestine and the pyloric caeca with little lipolytic activity in the stomach (Tocher, 2005). Many fish, including salmonids, lack a pancreas so they have a hepatopancreas that serves as the primary source of lipase enzymes. Fish also may secrete lipases from the intestinal mucosa (Tocher, 2005). There is evidence of a bile salt-activated lipase in fish that has high homology with its mammalian counterpart (Tocher, 2005). However, evidence for a pancreatic-lipase colipase system in fish is inconsistent. An enzyme similar to mammalian pancreatic lipase has been identified in rainbow trout, but it has low specific activity, low colipase activation, and requires bile salt for full activation (Tocher, 2005). In general, data suggests that lipolytic activity is primarily carried out by bile salt-activated lipases, but there could be a pancreatic-colipase system in some fish species as well. Nevertheless, the primary products of lipid digestion are free fatty acids and MAGs (Tocher, 2005). Lipid absorption, on the other hand, has not been well characterized in fish, but processes are assumed to be similar to that in mammals. Absorption proceeds at lower rates in fish compared to mammals because of lower body temperatures (Tocher, 2005). Transport of dietary lipids by chylomicrons, as described below, is considered to be the same in fish and in mammals. Likewise, fatty acid transport by lipoproteins, as described above, is similar between fish and mammals (Tocher, 2005).

Chylomicrons are synthesized in intestinal cells. The ApoB gene is transcribed in the nucleus. ApoB editing complex (APOBEC) then changes a CAA codon to UAA codon, a stop codon, causing the gene to be shortened creating a protein that is only 48% of the original length. ApoB48 is then translated in the rough endoplasmic reticulum (ER). Next, ApoB48 is added to the TAG and cholesterol droplet in the Golgi apparatus (Chow, 2008). ApoB48 transcription rate is constitutive and the rate of chylomicron formation is determined by the amount TAG present to be packaged. The primary role of ApoB48 is to make TAGs and cholesterol water soluble for transport. Chylomicrons are then released into the lymphatic system. Chylomicrons slowly move through the lymphatic system and enter the blood stream through the thoracic duct (Voet and Voet, 2004). It takes roughly three hours for dietary fat in chylomicrons to reach the blood stream. Once chylomicrons are in the blood stream, they bump into other molecules such as

HDL. When chylomicrons bump into HDL molecules, ApoCII/CIII and ApoE are transferred from HDL to the chylomicron (Chow, 2008). ApoE is required for interactions of chylomicrons with lipoprotein lipase (LPL); it tethers chylomicrons to endothelial cells through its interaction with heparin sulfate proteoglycan (HSPG). ApoE's interaction with HSPG holds the chylomicron in place while LPL hydrolyzes the TAGs (Lindberg and Olivecrona, 2002). The concentration of ApoCII/CIII determines the rate at which LPL hydrolyzes TAGs of chylomicrons. ApoCII stimulates LPL while ApoCIII inhibits LPL. The concentrations of ApoCII/CIII are determined by the liver because HDL, ApoCII, and ApoCIII are synthesized in the liver (Voet and Voet, 2004). With all of the apoproteins attached, the chylomicron becomes a substrate for LPL. LPL hydrolyzes TAGs of chylomicrons to free fatty acids and MAG. MAGs are then further hydrolyzed by MAG lipase (MAGL) into free fatty acids and glycerol. Free fatty acids are taken up by tissues and are used for energy or are stored; glycerol is taken up by the liver. Only TAGs are removed from chylomicrons (Chow, 2008). Once 85% of the TAGs have been hydrolyzed by LPL, ApoCII and CIII are recycled and are transferred back to HDL that the chylomicron comes into contact with. The chylomicron remnant now contains primarily dietary cholesterol, phospholipid, some TAG, ApoE, and ApoB48. The chylomicron remnant is taken up by the liver through receptor-mediated endocytosis (Chow, 2008). ApoE is required for endocytosis of the chylomicron. ApoE tethers chylomicron remnants through its interaction with HSPG to liver so hepatic lipase can hydrolyze the remaining fatty acids (Gibbons et al., 2000). The remnant is then transferred to the coated pit for endocytosis. There can also be LDL-receptor dependent endocytosis of the chylomicron remnant (Gibbons et al., 2000). The remnant binds LDL receptor-like protein and ApoE tethers the molecule through its interaction with HSPG. The remnant then undergoes receptor-mediated endocytosis. Cholesterol and remaining TAGs that were in the chylomicron are then packaged into VLDLs for further circulation through the blood stream. The cholesterol can also be used in bile synthesis while TAGs can be used for energy in the liver if necessary (Voet and Voet, 2004).

Receptor-mediated endocytosis requires multiple steps. First, the lipoprotein binds the receptor. Next, the chylomicron and receptor bud into the cell to form coated vesicles (Voet and Voet, 2004). The clathrin coats depolymerize as triskelions forming uncoated vesicles. The uncoated vesicles fuse with endosomes, which have an internal pH of 5.0. The acidity induces

the chylomicron to disassociate from the receptor (Voet and Voet, 2004). The receptors and chylomicrons accumulate in separate sections of the endosome and the receptor portion disassociates and recycles to the cell membrane. The remaining portion of the endosome fuses with a lysosome yielding a secondary lysosome wherein the lipoproteins are degraded into its component cholesterol esters, and amino acids (Voet and Voet, 2004). Cholesterol esters then are hydrolyzed to yield free cholesterol and fatty acids. This process is the same for all lipoproteins that undergo receptor-mediated endocytosis (chylomicrons, LDL, IDL) (Voet and Voet, 2004).

De novo Fatty Acid Synthesis: Fatty acid synthesis is considered to be highly conserved among and between species; the major difference is in the amount of synthesis. Fish generally undergo less *de novo* fatty acid synthesis because their diets are rich in fatty acids. Most of the lipid accumulated in fish is, therefore, mainly derived from the diet. However, fish are capable of modifying dietary fatty acids by further elongating or desaturating them (Tocher, 2005). Nevertheless, the *de novo* fatty acid synthesis pathway is essentially the same in fish and mammals. *De novo* fatty acid synthesis (Figure 3a) involves two main enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). ACC is the rate determining step (Voet and Voet, 2004). ACC catalyzes the cytosolic reaction that converts acetyl-CoA to malonyl-CoA. However, acetyl-CoA is produced in the mitochondria of the cell and cannot pass through the mitochondrial membrane; therefore acetyl-CoA is converted to citrate in the first step of the citric acid cycle (TCA) catalyzed by citrate synthase (Voet and Voet, 2004; Chow 2008). Citrate is transported into the cytoplasm and converted back to acetyl-CoA by ATP-citrate lyase which produces acetyl-CoA and oxaloacetate. The shuttle system is completed with two additional steps where oxaloacetate is converted to malate by malate dehydrogenase; malate is subsequently converted to pyruvate by malic enzyme (Voet and Voet, 2004). Pyruvate can pass through the mitochondrial membrane, back into the matrix. In the cytosol, acetyl-CoA is then converted to malonyl-CoA by ACC. ACC exists in two forms, the activated dephosphorylated form and the inactive phosphorylated form. Its hormonal regulation is described below.

FAS is the second enzyme involved in fatty acid synthesis. FAS catalyzes the formation of palmitic acid from seven acetyl-CoA molecules and malonyl-CoA (Voet and Voet, 2004; Chow, 2008; Ratnayake and Galli, 2009). FAS is a multifunctional enzyme that catalyzes seven

enzymatic reactions. Mammalian FAS functions as a dimer with an acyl carrier protein (ACP) phosphpantetheine chain that transports substrates between the various enzymatic domains (Voet and Voet, 2004). The first step of FAS is a priming step that transfers an acetyl group from acetyl-CoA to ACP to yield acetyl-ACP catalyzed by malonyl/acetyl-CoA-ACP transacylase (MAT). MAT also catalyzes the second step where malonyl-CoA replaces acetyl-CoA (Voet and Voet, 2004). The third step is the coupling of the acetyl group to the malonyl group forming acetoacetyl-ACP. The β carbon is then reduced with NADPH producing carbon dioxide and butyryl-ACP (Voet and Voet, 2004). Next, butyryl-ACP is reduced by β-ketoacyl-ACP reductase (KR), then dehydrated by β-hyrdoxyacyl-ACP dehydrase (DH), and then reduced again by enoyl-ACP reductase (EAR). The butyryl group from butyryl-ACP is transferred to the cysteine of ketosynthase (KS), elongating the initial priming acetyl-CoA molecule by two carbons (Voet and Voet, 2004). The ACP then binds another malonyl-CoA, and another cycle is repeated to add the next two carbon units to the chain. These steps (3-7) are repeated seven times to produce palmitoyl-ACP (Voet and Voet, 2004). The palmitoyl-ACP thioester bond is hydrolyzed by palmitoyl thioesterase (TE) producing palmitate, the final product of fatty acid biosynthesis (Voet and Voet, 2004).

Elongation and Desaturation: The end product of FAS, C16:0, is subject to elongation and desaturation (Figure 3a). There are two types of elongases, mitochondrial and ER-based. Both types extend the carbon chain by two carbons per reaction (Voet and Voet, 2004). Mitochondrial elongation is the reverse reaction of β-oxidation with successive addition and reduction of acetyl units (Voet and Voet, 2004). The difference is that the final reduction step involves NADPH as the terminal redox coenzyme instead of $FADH_2$. Elongation in the ER, on the other hand, condenses malonyl-CoA with acyl-CoA. The difference between this elongation and the FAS reaction is that the fatty acid is elongated as its CoA derivative, rather than its ACP derivative (Voet and Voet, 2004). There are several desaturases that insert double bonds into the carbon chain with no chain-length specificities. The major desaturases in animals are Δ^9-, Δ^6-, Δ^5-, Δ^4-fatty acyl-CoA desaturases (Ratnayake and Galli, 2009). In *de novo* fatty acid synthesis the first and most active desaturase is Δ^9-desaturase (stearoyl-CoA desaturase; SCD1). SCD1 inserts a double bond between carbons nine and ten to produce 16:1n-9 or 18:1n-9 (Ratnayake and Galli, 2009). These products can be further elongated into other monounsaturated fatty acids

(MUFA). The other desaturases have more of a role in synthesizing highly unsaturated fatty acids (HUFA) such as 20:4n-6 (ARA), 20:5n-3 (EPA), and 22:6n-3 (DHA) from 18:2n-6 (linoleic) and 18:3n-3 (α-linolenic) (Ratnayake and Galli, 2009). Most animals do not have Δ^{12}- or Δ^{15}-desaturases, but plants do. Therefore, animals are not able to synthesize 18:2n-6 or 18:3n-3 making them essential fatty acids that must be consumed in the diet (Ratnayake and Galli, 2009). In addition, 18:2n-6 and 18:3n-3 are important because they are required precursors for long-chain, n-6 and n-3 PUFAs. Both n-6 and n-3 PUFAs synthesis pathways are distinct with no cross reactions, but they do undergo the same alternating reactions employing the same enzymes to desaturate and elongate (Ratnayake and Galli, 2009) (Figure 3b). The first step employs Δ^6-desaturase followed by elongation and subsequent desaturation with Δ^5-desaturase to form 20:4n-6 and 20:5n-3. Δ^6-desaturase is the rate limiting step, and it has a higher affinity for 18:3n-3 compared to 18:2n-6 (Ratnayake and Galli, 2009). The next step involves two successive elongation steps and desaturation with Δ^6-desaturase to form 24:6n-3 (Ratnayake and Galli, 2009). The fatty acids then undergo one round of β-oxidation to yield 22:6n-3 (Ratnayake and Galli, 2009).

***Triglyceride Synthesis*:** Triglycerides consist of three fatty acids and a glycerol backbone synthesized from fatty acyl-CoA esters and glycerol-3-phophate (G3P) (Voet and Voet, 2004). The first step in TAG synthesis is catalyzed by glycerol-3-phosphate acyltransferase (GPAT) in the mitochondria or ER (Figure 3c) (Voet and Voet, 2004). The G3P used in the first step of TAG synthesis is derived from glucose via the glycolytic pathway or from oxaloacetate via gluconeogenesis (Voet and Voet, 2004). Freed glycerol from fatty acid β-oxidation is recycled and converted back to G3P by glycerol kinase; however, this reaction can only occur in the liver. GPAT converts G3P to a lysophosphatidic acid by adding a fatty acid to the R1 position (Voet and Voet, 2004). The lysophosphatidic acid is then converted to phosphatidic acid by 1-acylglycerol-3-phosphate acyltransferase, which adds a fatty acid in the R2 position. Phosphatidic acid can be converted to phospholipids or DAG; the latter reaction is catalyzed by phosphatidic acid phosphatase (Lipin1) which removes the phosphate group in the R3 position (Voet and Voet, 2004). Monoacylglycerol acyltransferase converts MAG to DAG by adding a fatty acid to the R2 position. DAG is then converted to TAG by diacylglycerol acyltransferase which adds a third fatty acid in the R3 position (Voet and Voet, 2004). DAG can also be

synthesized from MAG absorbed during intestinal lipid digestion. In general, the acyltransferases are not specific to particular fatty acids (Voet and Voet, 2004).

De novo Synthesized Fatty Acid Transport: Transport of *de novo* synthesized fatty acids and TAGs involves mainly the liver; synthesized TAGs are packaged in VLDL in the liver (Chow, 2008). VLDL synthesis is much like that of chylomicrons. ApoB is transcribed in the nucleus of cells in the liver and translated in the rough ER. The liver lacks ApoBEC that cuts ApoB down to ApoB48 in the intestine; therefore the liver produces lipoproteins with ApoB100 (Voet and Voet, 2004). The TAGs and cholesterol are added through the actions of the smooth ER. ApoB100 surrounds the TAG/cholesterol package in the Golgi apparatus. The resulting VLDL is then released directly into the blood stream (Figure 1) (Voet and Voet, 2004). The rate of VLDL synthesis is directly related to the amount of TAG available for packaging. Once in the bloodstream, VLDL bumps into HDL molecules, and ApoCII/CIII and ApoE are transferred from HDL to the VLDL (Voet and Voet, 2004). ApoE is required for the interaction of VLDL with LPL. ApoE tethers VLDL to endothelial cells through its interaction with HSPG. ApoE's interaction with HSPG holds VLDL in place while LPL hydrolyzes TAGs. The ratio of ApoCII/CIII determines the rate at which LPL hydrolyzes TAGs of the VLDL. ApoCII stimulates LPL while ApoCIII inhibits LPL (Voet and Voet, 2004). The concentrations of ApoCII/CIII are determined by the liver because HDL and ApoCII and ApoCIII are synthesized in the liver. With all apoproteins attached, VLDL becomes a substrate of LPL. LPL hydrolyzes TAGs to free fatty acids and MAG (Voet and Voet, 2004). As TAGs are hydrolyzed, the VLDL molecule becomes smaller and denser and becomes considered IDL. The apoproteins remain attached allowing IDL to be a substrate of LPL as well (Voet and Voet, 2004). As more TAGs are hydrolyzed, IDL becomes even denser and is then considered LDL. Once the molecule has lost enough of its TAGs to be considered LDL ApoCII/CIII and ApoE are recycled and are transferred to HDL (Voet and Voet, 2004). The resulting LDL only has ApoB100 along with its remaining TAG, cholesterol, and phospholipids. LDL is then cleared by the liver through receptor-mediated endocytosis with the LDL receptors binding LDL. If sufficient TAGs are removed from VLDL by LPL, VLDL can become LDL directly, skipping IDL.

Uptake of LDL is primarily dependent on the number of LDL receptors. The number of receptors is controlled transcriptionally by sterol regulatory element-binding protein (SREBP). There are two isoforms involved in lipid metabolism. SREBP1c is involved in the transcription of fatty acid synthesis genes such as ACC, FAS, and SCD1 (Voet and Voet, 2004). SREBP2 is involved in cholesterol synthesis and LDL receptor expression. SREBP1c is activated by insulin and inhibited by polyunsaturated fatty acids (PUFA) while SREBP2 is activated by sterols, especially cholesterol (Voet and Voet, 2004). Regardless of the isoform, the model for eliciting transcriptional regulation is the same and is illustrated in Figure 4. During basal conditions, when insulin is low and cholesterol is high, SREBP is in the membrane of the ER bound to SREBP cleavage-activating protein (SCAP). SCAP is the sensing portion of the complex. When cholesterol levels fall or insulin levels rise, SCAP transports SREBP to the Golgi apparatus where SREBP undergoes sequential proteolytic cleavage by site-1 protease (S1P) and site-2 protease (S2P) (Voet and Voet, 2004). This releases SCREP's dHLH/Zip-containing N-terminal domain. The dHLH portion then translocates into the nucleus of the cell, binds the sterol regulatory element (SRE), and increases transcription of its target genes (Voet and Voet, 2004). This process allows cholesterol or PUFA concentrations to depict the amount of LDL receptors on the cell's surface. Once the gene is transcribed and the protein is translated in the ER, the receptor is tranlocated to the cell membrane. Therefore, the number of LDL receptors increases when cholesterol or PUFA levels are low allowing for increased uptake of LDL. The LDL receptor-like protein, that is involved in the uptake of chylomicron remnants and some IDL molecules functions in much the same way. The rate of uptake is dependent on receptor availability, which is controlled transcriptionally through the SREBP/SCAP pathway (Voet and Voet, 2004). In addition, the transcription of several lipogenic genes is regulated through this same pathway.

Fatty Acid β-Oxidation: Like fatty acid synthesis, lipolysis and β-oxidation are highly conserved among speices and are considered to be the same in fish and mammals (Tocher, 2005). β-oxidation occurs in the mitochondria and is simply the breakdown of fatty acids into acetyl-CoA (Figure 5). In order for β-oxidation to occur, the fatty acid must first be primed by acyl-CoA synthetase, which forms a fatty acyl-CoA. The fatty acyl-CoA must then be transported into the mitochondria by carnitine palmitoyltransferase I (CPT1), the rate limiting

step in β-oxidation (Voet and Voet, 2004). CPT1 is located on the internal and external surfaces of the mitochondrial membrane. Translocation of the fatty acyl-CoA is mediated by a protein carrier that transports acyl-carnitine into the mitochondria while transporting free carnitine out (Voet and Voet, 2004). In this process CPT1 transfers the fatty-acyl group to carnitine releasing CoA. Fatty-acyl carnitine is then transported into the mitochondria by CP translocase as it subsequently transfers free carnitine out. Fatty-acyl carnitine is then converted back to fatty acyl-CoA and free carnitine by CPT2 located on the inner mitochondrial membrane. Free carnitine is transported out of the mitochondria by CP translocase, and the fatty acyl-CoA can enter the β-oxidation pathway (Voet and Voet, 2004).

β-oxidation breaks fatty acids down into two-carbon units of acetyl-CoA, much like fatty acid synthesis builds fatty acids by adding two-carbon units from acetyl-CoA. There are four steps that are repeated until the fatty acid is completely broken down into acetyl-CoA. Mammalian-derived fatty acids have an even number of carbons, while some plant-derived fatty acids have odd-chain lengths. Odd-chain fatty acids undergo the same β-oxidation process, but the final product is propinyl-CoA, made of three carbons, which must enter another pathway to be further broken down (Voet and Voet, 2004). The first step of β-oxidation involves dehydration by acyl-CoA dehydrogenase (ACDH) that inserts a trans-double bond between α and β carbons. The double bond is then hydrated by enoyl-CoA hydratase to form 3-L-hydroxyacyl-CoA (Voet and Voet, 2004). The third step is the NAD^+-dependent dehydrogenation by 3-L-hydroxyacyl-CoA dehydrogenase to form the corresponding β-ketoacyl-CoA. The fourth step is the cleavage of the Cα-Cβ bond by β-ketoacyl-CoA thiolase forming acetyl-CoA and a new fatty acyl-CoA with two less carbons (Voet and Voet, 2004). These four steps are repeated until all of the carbons have been broken down to acetyl-CoA.

Long-chained fatty acids undergo peroxisomal β-oxidation which is basically the same process, but it occurs in the peroxisome of the cell. There are, however, distinct differences in the enzymes involved in the two fatty acid oxidation pathways. The first enzymatic reaction is carried out by acyl-CoA oxidase (ACO) (Voet and Voet, 2004). It requires a FAD cofactor but it transfers electrons directly to oxygen rather than passing them through the electron transport chain. This mechanism makes peroxisomal β-oxidation less efficient than mitochondrial β-

oxidation because it produces two less ATPs per two-carbon cycle (Voet and Voet, 2004). The resulting H_2O_2 is deprotonated by peroxisomal catalase producing H_2O and O_2. The remaining β-oxidation reactions are identical to the mitochondrial reactions, but are catalyzed by a peroxisomal bifunctional protein, enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase enzyme (EHHADH) (Voet and Voet, 2004). The acyl-CoA dehydrogenases used in β-oxidation vary depending on the length of the fatty acid chain. There are medium-chain acyl-CoA dehydrogenase (ACDHM) and very long-chain acyl-CoA dehydrogenase (ACDHVL) (Ratnayake and Galli, 2009). Peroxisomes, therefore, do not completely oxidize fatty acids because they cannot oxidize fatty acids with fewer than eight carbons. The shorter-chained fatty acids are transported to the mitochondria for complete oxidation and the resulting acetyl-CoA can then enter the TCA cycle; resulting NADH + H^+ donates its electrons to the electron transport chain for oxidative phosphorylation (Voet and Voet, 2004). For odd chain fatty acids, the resulting propionyl-CoA is converted to succinyl-CoA which is an intermediate of the TCA cycle (Voet and Voet, 2004).

Fatty acid β-oxidation is regulated in two ways: 1) regulation of CPT1 and 2) the rate of lipolysis. CPT1 is primarily regulated by malonyl-CoA. Malonyl-CoA is the product of the ACC reaction converting acetyl-CoA to malonyl-CoA, the rate limiting step of fatty acid biosynthesis (Voet and Voet, 2004). When malonyl-CoA levels are high because of stimulated fatty acid synthesis, CPT1 is allosterically inhibited to prevent β-oxidation from occurring at the same time as fatty acid synthesis (Voet and Voet, 2004). The rate of malonyl-CoA formation is controlled by insulin. When blood glucose levels are high and insulin is in circulation, insulin causes dephosphorylation of ACC through its actions via its tyrosine kinase receptors. Therefore, insulin indirectly inhibits β-oxidation by stimulating the formation of its rate limiting enzyme's inhibitor. When malonyl-CoA is not present, β-oxidation is not inhibited (Voet and Voet, 2004).

Lipolysis: The second way β-oxidation is regulated is through its supply of fatty acids provided by lipolysis (Figure 5). To mobilize TAGs and fatty acids from an adipocyte lipid droplet, a series of phosphorylations of several proteins must occur. First, the lipid droplet is surrounded by a protein called perilipin that protects the TAG from lipases. During the fasted state, glucagon causes the phosphorylation of perilipin through its activation of cAMP (Voet and

Voet, 2004; Carmen and Victor, 2006). Once phosphorylated, perilipin allows the translocation of lipases to begin, hydrolyzing TAGs to free fatty acids (Voet and Voet, 2004). Insulin, on the other hand, stimulates dephosphorylation of perilipin by phosphatases and therefore prevents lipolysis and β-oxidation. The rate limiting enzyme of lipolysis is hormone sensitive lipase (HSL) (Voet and Voet, 2004). HSL is inactive when dephosphorylated and active when phosphorylated. Insulin stimulates the dephosphorylation of HSL by activating phosphatases (Voet and Voet, 2004). This therefore prevents lipolysis from occurring and thereby prevents β-oxidation. During the fasted state glucagon stimulates the phosphorylation of HSL through the activation of its kinases (Carmen and Victor, 2006). This activation of HSL along with the phosphorylation of perilipin allows for lipolysis and therefore increases β-oxidation in order to supply energy (Voet and Voet, 2004). Once lipases translocate into the lipid droplet, adipose TAG lipase (ATGL) and HSL reduce TAGs to MAGs, and two free fatty acids are then released into the cytosol (Carmen and Victor, 2006). TAG lipase hydrolyzes the fatty acid in the R_3 position. HSL then hydrolyzes the fatty acid in the R_1 position (Voet and Voet, 2004). The resulting MAG is released into the cytosol where MAGL removes the final fatty acid in the R_2 position yielding the glycerol backbone (Voet and Voet, 2004). Glycerol is readily diffusible and goes to the liver to be converted to G3P by glycerol kinase. This reaction cannot occur in adipose tissue because it does not have the glycerol kinase enzyme (Voet and Voet, 2004).

Non-Esterified Fatty Acid Transport: Very little fatty acid oxidation occurs in adipose tissue because it requires little energy, therefore freed fatty acids must be transported to tissues with mitochondria for β-oxidation and energy production. Since fatty acids are insoluble, they must be transported. During lipolysis, fatty acids are transported through the blood by albumin, not lipoproteins (Figure 5) (Voet and Voet, 2004). Each albumin molecule can carry eight fatty acids. Once albumin reaches a target cell, fatty acids are transported into the cell by fatty acid translocase/cluster of differentiation 36 (FAT/CD36), but these fatty acids must be bound to fatty acid binding proteins (FABP) or be metabolized immediately because micelles cannot be allowed to form in the cell (Ratnayake and Galli, 2009). There are several types of FABPs that are tissue dependent. The main ones of concern are FABP1 in the liver, FABP2 in the intestine, FABP3 in muscle and heart, and FABP4 in adipocytes (Chmurzynska, 2006).

Acetyl-CoA as a Metabolic Intermediate: Acetyl-CoA is a metabolic intermediate that is an important branch point. Acetyl-CoA can feed into three pathways: 1) TCA cycle, 2) fatty acid synthesis, and 3) ketone body formation. Acetyl-CoA enters the TCA cycle at the citrate synthase step where oxaloacetate (OAA) is converted to citrate (Voet and Voet, 2004). The rate limiting step in the TCA cycle is the conversion of isocitrate to α-ketoglutarate by isocitrate dehydrogenase. The TCA cycle is responsible for creating 28 ADP, 10 NADH, 2 $FADH_2$ and 34 P_i molecules that feed into the electron transport chain for oxidative phosphorylation (Voet and Voet, 2004). Oxidative phosphorylation converts those molecules to 28 ATP, 10 NAD^+, and 2 FAD molecules. These processes occur under aerobic conditions (Voet and Voet, 2004). Acetyl-CoA is also used in the fatty acid biosynthesis pathway; which was previously discussed. A third pathway acetyl-CoA can enter is ketone body formation. This pathway is activated when acetyl-CoA is being produced from β-oxidation faster than the TCA cycle and oxidative phosphorylation can use it (Voet and Voet, 2004). Ketogenesis primarily occurs in liver mitochondria where acetyl-CoA is converted to acetoacetate, D-β-hydroxybutyrate, or acetone. These compounds serve as metabolic fuels for peripheral tissues during bouts of starvation (Voet and Voet, 2004). Ketone bodies are synthesized in three enzymatically catalyzed reactions. The first reaction is catalyzed by acetyl-CoA acetlytransferase (ACAT) which catalyzes the reverse reaction of the final step in β-oxidation combining two acetyl-CoA molecules to form acetoacetyl-CoA (Voet and Voet, 2004). A third acetyl-CoA is added by HMG-CoA synthase to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). The cleavage of HMG-CoA into acetyl-CoA and acetoacetate is then catalyzed by HMG-CoA lyase (Voet and Voet, 2004).

Hormonal Control of Fatty Acid Metabolism:

Insulin and Glucagon: Most mammals eat meals and go hours between meals. To prevent alternation of feast and famine at the cellular level, there are mechanisms in place to help balance nutrient availability. These mechanisms ensure storage of nutrients directly after a meal and mobilization of nutrient stores between meals. There are two primary hormones that elicit these effects on nutrient metabolism, insulin and glucagon. Both hormones respond to blood glucose levels; insulin is released when glucose levels are high, while glucagon is released when blood glucose levels are low. In general, insulin decreases blood glucose levels through

activating storage of carbohydrates, lipids, and amino acids by increasing their synthesis and uptake in muscle and adipose tissue (Voet and Voet, 2004).

Insulin is the only hormone in the body that can stimulate the uptake of glucose. Glucose uptake in muscle and adipose tissue cells is insulin-dependent, whereas glucose uptake in the brain and liver is insulin-independent. In insulin-dependent cells insulin lowers blood glucose by directly acting on cells to increase glucose uptake by increasing the number of type 4 glucose transports (GLUT4) present on the cell membranes. Insulin also increases glucose-using pathways while decreasing endogenous fuel-producing pathways to help lower blood glucose concentrations (Voet and Voet, 2004). Insulin elicits its effects on these pathways in a receptor-mediated manner through a tyrosine kinase receptor. The insulin receptor acts as an $\alpha_2\beta_2$ tetramer (Figure 6a). The α subunits contain the extracellular binding site and the β subunits anchor the receptor in the membrane of the cell and contains the tyrosine kinase domain that elicits the enzymatic activity of the receptor (Voet and Voet, 2004). When insulin binds the α subunits it activates the tyrosine kinase activity of the β subunit which auto-phosphorylates the tyrosine residues of the β subunits. The activated tyrosine kinase then phosphorylates second messenger proteins such as insulin receptor substrate-1 (IRS-1). Once IRS-1 is phosphorylated it acts as a docking protein for other protein messengers that mediate the actions of insulin (Voet and Voet, 2004). One protein that is activated by its phosphorylation is phosphatidylinositol-3-kinase (PI3K). IRS-1 causes reactions that convert phosphatodylinosityl-4,5-diphosphate (PIP2) to become phosphatodylinosityl-3,4,5-triphosphate (PIP3). This activates Akt. Akt stimulates the movement of GLUT4 transporters to the cell's membrane. It also activates protein phosphatase-1 (PP-1) which dephosphorylates proteins in nutrient utilization pathways regulated by phosphorylation, such as fatty acid synthesis and β-oxidation. Lastly, it inactivates glycogen synthase kinase-3 (GSK3) through the activation of protein kinase c (PKC). This decreases GSK3 activity by increasing its phosphorylation (Voet and Voet, 2004). Another group of second messengers activated by insulin tyrosine-kinase receptors are in the Cbl-P messenger system. This system facilitates the movement of GLUT4 transporters into the plasma membrane as well (Voet and Voet, 2004). Gene expression can also be altered by insulin through the activation of the RAS/Mek pathway that activates a series of MAP kinase cascade reactions that

controls gene expression of target genes (Voet and Voet, 2004). Overall, this signalling pathway results in increased glucose uptake and increased glycogen, lipid, and protein synthesis.

Glucagon acts through stimulating a G_s receptor (Figure 6b). The G_s receptor is a G-protein receptor that has seven trans-membrane helices and three subunits, α, β, and γ. When not stimulated, the α, β, and γ subunits are bound together and the α-subunit has GDP attached (Voet and Voet, 2004). Upon stimulation of the G_s receptor by glucagon, the α-subunit hydrolyzes a GTP to GDP, and its attached GDP becomes GTP. This conversion of GDP to GTP causes the α-subunit to disassociate from the β/γ-subunit complex (Voet and Voet, 2004). The activated α-subunit then activates adenylate cyclase (AC). AC then increases cyclic AMP (cAMP) concentrations which activates phosphoprotein kinases. The activated kinases then phosphorylate proteins and enzymes (Voet and Voet, 2004). The increased phosphorylation generally activates enzymes involved in gluconeogenesis as well as regulated enzymes within the lipolysis, β-oxidation, protein degradation, amino acid deamination, and glycogen degradation pathways. Conversely, phosphorylation will inhibit the regulated enzymes within glycolysis, fatty acid synthesis, protein synthesis, and glycogen synthesis pathways (Voet and Voet, 2004).

De novo fatty acid synthesis involves two main enzymes, ACC and FAS; both enzymes are regulated by insulin (Voet and Voet, 2004). ACC catalyzes the cytosolic reaction that converts acetyl-CoA to malonyl-CoA. ACC exists in two forms, the active dephosphorylated form and the inactive phosphorylated form. Insulin's activation of phosphoprotein phosphatase 2A (PP2A) causes ACC to be dephosphorylated and activated (Voet and Voet, 2004). Glucagon, on the other hand, activates AMP-dependent protein kinase (AMPK) which phosphorylates ACC, deactivating it (Voet and Voet, 2004). Phosphorylation is catalyzed differently in the liver than in other tissues. In the liver only AMPK phosphorylates ACC while in other tissues ACC is phosphorylated by AMPK and PKA. Both kinases are activated through glucagon's interaction with its receptor (Voet and Voet, 2004). ACC also exists in two mammalian isoforms, α-ACC and β-ACC. The α-ACC isoform only exists in adipose tissue and β-ACC is found in tissues that oxidize but do not synthesize fatty acids. The liver contains both isoforms. The β-ACC isoform's main function in non-synthesizing tissues is to produce malonyl-CoA because malonyl-CoA is a primary inhibitor of fatty acyl-CoA transport into the mitochondria, the rate limiting step for

fatty acid β-oxidation. Therefore β-ACC has a regulatory function in fatty acid oxidation (Voet and Voet, 2004).

Fatty acid synthase is the second enzyme involved in fatty acid synthesis. FAS catalyzes the formation of palmitic acid from acetyl-CoA and malonyl-CoA. Like several other enzymes involved in synthesis pathways that synthesize energy storage molecules, insulin controls FAS gene transcription, and therefore controls the amount of the enzyme available to catalyze fatty acid synthesis (Voet and Voet, 2004). Like the other genes, there is an insulin response unit (IRU) on the FAS gene. The IRU is subject to regulation by phosphorylation or dephosphorylation caused by downstream effectors of the insulin signally pathway (Voet and Voet, 2004). For FAS, insulin activates PI3K which in turn activates PKB. PKB and its downstream effectors are responsible for activating the IRU of the FAS gene. Therefore, when insulin is present FAS gene transcription is increased (Voet and Voet, 2004). When fasting, glucagon has opposite effects on FAS gene transcription by inhibiting the IRU activation. There is evidence the presence of glucose at high levels further enhances increases of FAS gene transcription increased by insulin (Voet and Voet, 2004). In general, when animals are fasted and then re-fed with a high carbohydrate diet, blood glucose concentrations are high and there is at least a four-fold increase in FAS gene transcription and a subsequent increase in the flux through the fatty acid biosynthesis pathway.

Fatty acid β-oxidation is regulated in two ways: 1) regulation of CPT1 and 2) the rate of lipolysis. Insulin indirectly inhibits β-oxidation by stimulating the formation of malonyl-CoA, CPT1's inhibitor. CPT1, the rate limiting enzyme of β-oxidation, is primarily regulated by malonyl-CoA. Malonyl-CoA is the product of the ACC reaction converting acetyl-CoA to malonyl-CoA, the rate limiting step of fatty acid biosynthesis (Voet and Voet, 2004). Insulin's and glucagon's actions on ACC, therefore, regulate CPT1 activity through allosteric regulation. The second way β-oxidation is regulated is through its supply of fatty acids provided by lipolysis. Glucagon regulates lipolysis through phosphorylating and activating perilipin and HSL. Perilipin must be phosphorylated in order to allow lipase's to enter the lipid droplet and HSL must be phosphorylated to hydrolyze fatty acids (Voet and Voet, 2004).

Overall, regulation of nutrient metabolism by insulin is complex. In general, after a high carbohydrate diet when blood glucose levels are high, insulin becomes the governing hormone. Insulin's main goal is to lower blood glucose concentrations. Under the direction of insulin, metabolic pathways that synthesize molecules for energy storage are activated. These activated pathways include glycolysis, glycogen synthesis, protein synthesis, and fatty acid synthesis. These pathways are activated by dephosphorylation of their regulatory enzymes which is caused by insulin's interaction with tyrosine kinase receptors (Voet and Voet, 2004). In contrast, pathways that breakdown energy storage molecules into glucose are inhibited by insulin. These inhibited pathways include gluconeogenesis, glycogen breakdown, and fatty acid β-oxidation. These pathways are therefore inhibited by dephosphorylation (Voet and Voet, 2004). This complex regulation of these pathways by insulin, along with its counterpart glucagon, is vital to maintaining healthy blood glucose concentrations. When there is a malfunction within these pathways, it can be detrimental to the cell's and organism's life. A list of regulated enzymes is provided in Table 3. The specific actions of insulin on metabolism are illustrated in Figure 6.

Growth Hormone and IGF-1: In all vertebrates, growth hormone (GH) is synthesized in the anterior pituitary, but its release is controlled by tropic factors released by the hypothalamus. Also, GH actions are not all direct, most of its actions on peripheral tissues are indirect through growth factors produced and released by the liver (Voet and Voet, 2004). Upon stimulation, the hypothalamus releases growth hormone releasing hormone (GHRH). GHRH then acts on the anterior pituitary, stimulating it to release GH. GH, in turn, acts on tissues (primarily the liver) causing them to synthesize and release insulin-like growth factor-1 (IGF1; somatomedin). IGF1 acts to maintain appropriate levels of circulating GH by participating in negative feedback loops to the pituitary and hypothalamus (Voet and Voet, 2004). Similarly, GH and GHRH act through negative feedback loops on the hypothalamus to inhibit GH release. When IGF1, GH, or GHRH levels are high, the hypothalamus releases somatostatin which inhibits the pituitary from producing more GH (Gerrard and Grant, 2006). Moreover, IGF1 mediates many of GH's effects on other tissues including bone, muscle, and adipose tissue. In the bone, IGF1 increases chondrocyte proliferation and osteoblast differentiation leading to increased bone lengthening prior to epiphyseal closure and increased bone mass through periosteal growth (Gerrard and Grant, 2006). GH and IGF1 increase lean muscle growth by increasing protein synthesis while

decreasing protein degradation. GH can act either directly by stimulating myogenic cells to release IGF1 or through IGF1 release from the liver (Gerrard and Grant, 2006). In addition, GH and IGF1 increase lipolysis and decrease lipogenesis in adipose tissue.

GH secretion is influenced by a number of factors including exercise, stress, low levels of glucose and fatty acids, and high levels of amino acids in the blood (Gerrard and Grant, 2006). One of the most influential factors is the circadian rhythm. GH levels are relatively low and constant. GH levels rise during the night in mammals and in rainbow trout (Gelineau et al., 1996). In salmonids, such as rainbow trout, GH and IGF1 levels rise during increased day-length hours (summer months) and elevated water temperatures. Additionally, elevated levels of cortisol during bouts of stress will increase the release of GH (Flores et al., 2012). GH levels also rise just after a feeding event and remain elevated for roughly 8 hours after feeding in juvenile rainbow trout (Gelineau et al., 1996). There is a rise in corresponding IGF1 levels during the summer months (June-October) in rainbow trout (Taylor et al., 2008). Conversely, there does appear to be a positive correlation between pituitary GH, mRNA concentrations or plasma GH levels and gonadogenesis (Gomez et al., 1998). Sex steroids are known to increase the release of GH and IGF1. During sexual maturation in female rainbow trout, there is an increase in estrogen that increases the release of GH. In turn, GH stimulates lipolysis and energy production to support increased energy demands of gonadogenesis and vitellogenesis. In general, GH mRNA levels in the pituitary remain constant during vitellogenesis, but there is a significant increase in GH mRNA concentrations during oocyte maturation (stage 5). GH levels within the pituitary increased during exogenous vitellogeneis throughout oocyte maturation (stage 3-stage 5). Although plasma GH levels were not significantly different throughout the stages of oogenesis ($P<0.05$), there was a trend for plasma GH levels to decrease during oogenesis ($P=0.053$) (Gomez et al., 1998). These findings suggest that GH release is stimulated when sex steroid levels reach their highest concentrations during late oogenesis. This hormonal relationship is also consistent with trout limiting their feed intake during sexual maturation; increased GH concentrations during and after ovulation suggest a role for GH in redirecting energy toward somatic growth from gonadal growth. This rise in GH concentrations after ovulation also occurs during the transition into summer when day length becomes longer.

Growth hormone also acts as an antagonist to insulin signaling, especially in adipose tissue. Growth hormone reduces the number of GLUT4 transporters in adipose tissue and decreases lipogenesis while increasing lipolysis. These effects cause muscle metabolism to shift and rely more on non-esterfied fatty acids (NEFA) rather than glucose for energy (Hocquette et al., 1998). In fish, growth hormone is believed to be involved in the shift in development from somatic tissue accretion to gonadal growth which leads to a reduction in body weight and condition (Sumpter et al., 1991). Sumpter et al. (1991) reported that the onset of gonadal growth leads to nutritional insufficiency resulting in lower condition and cessation of somatic growth. These changes during spawning season cause plasma growth hormone concentrations to increase. Increased plasma growth hormone concentration allows fish to regain somatic growth and increase condition after spawning (Sumpter et al., 1991). In 1991, Sumpter et al. determined that plasma growth hormone does not trigger maturation or gamete release, nor is growth hormone required for rapid somatic or gonadal growth. Triploid females maintain a low plasma growth hormone concentration and high condition throughout development (Sumpter et al., 1991). Most changes in adipose tissue during spawning are thought to be associated with nutritional insufficiency because they eat less while undergoing gonad development and maturation. Furthermore, Sumpter et al. (1991) suggested that nutritional insufficiency during reproduction causes decreases in condition factor associated with depleted intramuscular fat as the fat is mobilized from the muscle to support egg growth.

Estrogen: Estrogen elicits its effects on lipid metabolism through estrogen receptor α (ERα). Estrogen administration decreases adipocyte size and number in cultured mouse adipocytes. The decreased size of adipocytes is a result of estrogen inducing lipolysis. Estrogen acts through ERα receptor to increase gene expression of adipose triglyceride lipase (ATGL) and perilipin (Wend et al., 2013). Expression of ATGL in ERα knockout mice is decreased relative to the wild type expression and estrogen administration does not induce changes in ATGL expression in ERα knockout mice (Wend et al., 2013). Estrogen administration decreases perilipin protein content in wild type and ERα knockout mice. ERα knockout mice did maintain a higher level of perilipin protein when compared to the wild type mice regardless of treatment (Wend et al., 2013). Perilipin gene expression was, however, significantly lower in ERα knockout mice compared to wild type mice. Furthermore, estrogen treatment increased perilipin

mRNA, but had no effect in ERα knockout mice (Wend et al., 2013). These findings suggest estrogen regulates perilipin post-transcriptionally while regulating ATGL through altered gene expression, through ERα. Increased expression of ATGL facilitates increased lipolysis. ATGL is the first step in lipolysis, catalyzing the hydrolysis of TAG to DAG (Voet and Voet, 2004). Increased hydrolysis of TAG reduces lipid droplet size within adipocytes. Altered expression of perilipin can also affect the amount of lipid within adipocytes because it regulates lipolysis by controlling translocation and activation of lipases, mainly HSL (Voet and Voet, 2004).

Testosterone: Testosterone is a hormone with numerous physiological functions. Several studies have shown that testosterone's effects on fat-free mass are directly correlated to serum testosterone concentrations; whereas, changes in whole-body and regional fat mass are inversely correlated with testosterone levels in mammals (De Maddalena et al., 2012). There is an increased visceral adipose tissue deposition in hypogonadal individuals. Increased adipose tissue causes a further decrease in circulating testosterone levels through conversion of testosterone to estrogen by aromatase (De Maddalena et al., 2012). Testosterone inhibits adipogenic differentiation of preadipocytes by activating AR/β-catenin interaction leading to its translocation into the nucleus and subsequent down-regulation of adipogenic transcription factors (De Maddalena et al., 2012). Furthermore, serum concentrations of leptin are inversely related to testosterone levels. Leptin is primarily expressed in adipose tissue and acts to reduce appetite and increase energy expenditure. Testosterone decreases expression of leptin in cultured adipocytes (De Maddalena et al., 2012). Additionally, low testosterone levels are correlated with reduced levels of circulating ghrelin. Ghrelin is a gastric hormone that increases appetite and slows down metabolism (De Maddalena et al., 2012). In general, testosterone decreases adipogenesis by inhibiting adipocyte differentiation and reduces adipocyte size by decreasing lipogenesis and energy consumption.

Peroxisome Proliferator-Activated Receptor Signaling Pathway:

Lipids serve as primary sources of energy as well as potent regulators of metabolism by controlling metabolism through transcription regulation. One signaling pathway lipids induce to elicit control over transcription is the peroxisome proliferator-activated receptors (PPAR) pathway. PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors (Poulsen et al., 2012). PPARs alter transcription of genes involved in a variety of biological processes including development, reproduction, inflammation, immune function, metabolism, apoptosis, growth, and cancer (Poulsen et al., 2012). There are three subtypes of PPARs that have been identified: PPARα, PPARβ/δ, and PPARγ. The three isoforms have a high degree of amino acid homology in the molecular domains that bind DNA and ligands; however, they display distinct ligand specificity and DNA-binding sites (Abbott, 2009). Each receptor has an N-terminal "A/B" domain that has a ligand-independent activation function and it is poorly conserved between the various isoforms. The "C" domain encodes for the DNA binding region of the receptor and is highly conserved among the isoforms (Varga et al., 2011). The C-terminal ligand binding domain, the "E/F" domain, is responsible for ligand-dependent activation and is important for RXR (retinoid X receptor) heterodimerization (Varga et al., 2011). In general, all isoforms act through the same general mechanisms to elicit their individual effects. PPAR forms a heterodimer with RXR, another member of the nuclear receptor super family. The complex then binds a specific DNA sequence, peroxisome proliferator response element (PPRE), in the promoter region of target genes. In order for RXR to heterodimerize it must be activated by its ligand 9-cis-retinoic acid (Hausman et al., 2008). Once the complex interacts with the PPRE a series of co-activators are recruited to induce transcription of the target gene. Co-repressors may also be bound to prevent transcription of the target gene (Varga et al., 2011). An outline of PPARs actions on gene regulation is in Figure 7.

PPARα: All three PPAR subtypes are highly expressed in tissues involved in lipid metabolism; however, they have distinct expression profiles and biochemical properties resulting in subtype-specific activation of target genes (Poulsen et al., 2012). PPARα induces fatty acid oxidation and is highly expressed in tissues with substantial mitochondrial and peroxisomal β-oxidation, such as brown adipose tissue, liver, kidney, and heart (Poulsen et al., 2012). However, PPARα has low expression in white adipose tissue (Varga et al., 2011). Disruption of PPARα

prevents β-oxidation; however, adipose tissue develops normally (Hossner, 2006). In muscle, PPARα activation not only causes increased fatty acid oxidation, it decreases glucose uptake and induces mild insulin resistance. In the liver, PPARα is activated during the fasting state to increase expression of genes involved in fatty acid catabolism and ketogenesis. There is also evidence it decreases expression of genes associated with lipogenesis and fatty acid elongation in a sterol regulatory element binding protein (SREBP) manner. In general, PPARα induces fatty acid handling within the liver to either catabolize or store fatty acids, thereby diminishing cytotoxic effects of free fatty acids (Paulsen et al., 2012). PPARα increases expression of β-oxidation genes such as *cpt1, cpt2, acdh, ehhadh, acat2, magl,* and *aco* as well as transporters such as *cd36, lpl,* and *fabp* (Mandard et al., 2004; Labar et al., 2010). PPARα's primary function within adipose tissue appears to be activation of thermogeneic programs including activation of uncoupling protein-1 and PPARγ co-activator 1α gene expression (Pouslen et al., 2012).

PPARβ: PPARβ is ubiquitously expressed and has a general role is activating β-oxidation. It is known to increase endurance, oxidative myofiber switch, and glycogen storage along with increasing β-oxidation when activated in skeletal muscle (Poulsen et al., 2012). PPARβ primarily governs hepatic glucose utilization and lipoprotein metabolism in liver by mainly altering the gene expression of VLDL (Pouslen et al., 2012). PPARβ is expressed in many different tissues, but is not highly expressed in adipose tissue (Varga et al., 2011). It does, however, appear to play a role in priming preadipocytes for differentiation (Pouslen et al., 2012). PPARβ has also been linked to colon cancer and is known to down regulate the expression of PPARγ (Zuo et al., 2007).

PPARγ: PPARγ is highly expressed in adipose tissue, but has low expression in other tissues. PPARγ signals lipid accumulation and it is a key regulator of gene expression for adipose tissue development and adipocyte differentiation (Pouslen et al., 2012; Varga et al., 2011; Hossner, 2006). Activation of PPARγ leads to increased TAG accumulation in muscle and liver (Pouslen et al., 2012). It is the most abundant of the PPAR, occurring in adipose tissue at levels 30-fold higher than levels found in other tissues (Varga et al., 2011). PPARγ also increases insulin sensitivity, but is primarily considered the master regulator of adipocyte differentiation and adipocyte metabolism (Hossner, 2006). In general, PPARγ activation induces adipocyte

differentiation for both brown and white adipose tissue. There are two main variants of PPARγ, PPARγ1 and PPARγ2 in mammals. These isoforms are a result of alternative splicing of mRNA and alternate promoter sites of the PPARγ gene (Varga et al., 2011). PPARγ2 has 30 additional N-terminal amino acids distinguishing it from PPARγ1 (Hossner, 2006). There is also a third promoter in the human sequence coding for a third protein, PPARγ3 (Varga et al., 2011). Co-activators known to interact with the PPARγ complex include: steroid receptor co-activator 1 (SRC-1), CREB binding protein (CBP/p300), PPARγ co-activator-1 (PGC-1) and PGC-2, PPAR binding protein (PBP/TRAP220/DRIP230), and androgen receptor associated with protein 70 (ARA70). PPARγ elicits effects on a variety of target genes such as *fabps, cd36, lpl, leptin, fas, acc,* and *scd1* (Hossner 2006). The ligand binding domains are highly conserved, with rat and mouse PPARγ1 and γ2 having 95-98% homology with the human receptors (Varga et al., 2011).

Several ligands are known to interact with PPARγ. Hormones and growth factors such as insulin, growth hormone, and thyroid hormones are known to interact with PPARγ (Chung and Johnson, 2008). A variety of fatty acids and fatty acid derivatives (such as conjugated-linoleic acid (CLA), eicosanoids and prostaglandins) are known to elicit effects through the PPARγ signaling pathway (Smith et al., 2009; Duplus et al., 2000; Dodson et al., 2010; Hausman et al., 2009). In addition, micronutrients, such as vitamins A, C, and D, zinc, chromium, and magnesium are known to elicit effects on adipogenesis through the PPARγ signaling pathway (Kawachi, 2006; Gorocica-Buenfil et al., 2007). These ligands have varying effects on adipocytes and adipogenesis. Fatty acids can either up-regulate or down-regulate gene expression. Polyunsaturated fatty acids (PUFA), such as linoleate (C18:2n-6), down regulate expression of *fas. me,* and *glucose-6-phosphate* (*g6p*) dehydrogenase (Duplus et al., 2000). Both saturated (SFA) and unsaturated fatty acids are known to stimulate expression of *acyl-CoA oxidase* (*aco*), *L-fatty acid binding protein (l-fabp),* and *cpt1* (Duplus et al., 2000). SFA increases transcription of *ldl* and enzymes involved in fatty acid chain elongation (Viscarra and Ortiz, 2013). A low MUFA:SFA ratio would maintain lipolysis without increasing oxidation, allowing for preservation of energy stores to support energetic demands associated with food deprivation (Viscarra and Ortiz, 2013). CLA administration to weanling pigs reduces adipocyte volume and depresses *scd1* gene expression causing a decrease in monounsaturated fatty acids (MUFA) synthesis (Hausman et al., 2009). In rodents, CLA prevents lipid filling of adipocytes by

decreasing *pparγ* gene expression in preadipocytes (Brown et al., 2003). Vitamin A and D supplementation depresses adipogenesis while vitamin C supplementation increases adipocyte differentiation in beef cattle through interactions with PPARγ (Smith et al., 2009).

Ligands that affect adipogenesis can be used in meat-animal production to improve carcass quality (Hausman et al., 2009; Gorocica-Buenfil et al., 2007; Smith et al., 2009; Dodson et al., 2010). In the United States there has been a downward trend in cattle that grade Choice (Smith et al., 2009). This trend is largely due to loss of corn and other grains to the production of ethanol (Smith et al., 2009). Feed manipulation, hormone supplementation, and selective breeding have been primary methods used to improve animal growth, but these methods decrease muscle quality by decreasing the amount of fat on the carcass (Dodson et al., 2010). These decreases in muscle quality have stimulated an interest in developing alternative technologies to alter lipid deposition and selectively enhance intramuscular fat deposition in meat animals (Dodson et al., 2010). However, mechanisms leading to differential lipid accumulation in visceral, subcutaneous, intermuscular, and intramuscular fat depots remain unclear (Dodson et al., 2010). PPAR ligands, especially PPARγ, show the most promise in manipulating adipose tissue development by altering adipogenesis.

Post-translational Modifications: In addition to PPAR ligand-mediated activation, PPAR activity is also modified by phosphorylation, which affects their action in both a ligand-dependent and ligand-independent manner (Diradourian et al., 2004). Phosphorylation, therefore, provides a mechanism to alter the activity of PPARs that is dependent on other signaling pathways providing a mechanism for crosstalk between pathways. Phosphorylation of PPARs has varying affects dependent on the serine phosphorylated and PPAR subtype. PPARα and PPARγ A/B domains are phosphorylated, but PPARβ is not (Diradourian et al., 2004; Bugge and Mandrup, 2010). The ERK/MAPK pathway is the primary pathway leading to serine phosphorylations altering PPAR transcriptional activity. MAPK-phosphorylation of serine 12 and 21 of PPARα increases transcriptional activity by increasing receptor stability through decreased ubiquitination. Increased PPARα activity by phosphorylation in rat hepatocytes increases subsequent expression of genes within the β-oxidation pathway. On the other hand, phosphorylation of seine 76 of PPARα by glycogen synthase kinase increases ubiquitination and

degradation (Buggie and Mandrup, 2010). MAPK-phosphorylation of serine 82 of PPARγ1 inhibits its activity by decreasing the ligand-binding affinity of the receptor (Diradourian et al., 2004; Bugge and Mandrup, 2010). Whereas MAPK-phosphorylation of serine 112 (the corresponding serine to serine 82 of PPARα) of PPARγ2 increases transcriptional activity of PPARγ. This suggests that the cellular and molecular context determines the transcriptional effects of PPARγ, A/B domain phosphorylation. Furthermore, serine 16 or 21 phosphorylation of PPARγ by casein-kinase II promotes shuttling of PPARγ from the nucleus to the cytosol, inhibiting PPARγ actions on lipogeneic gene transcription (Bugge and Mandrup, 2010). In general, phosphorylation of PPARβ has been less studied; however, there is evidence that cAMP and PKA are involved in inhibition of PPARβ actions either through phosphorylation of PPARβ directly or by affecting interaction of PPARβ with its coactivators or corepressors (Diradourian et al., 2004).

Phosphorylation can alter actions of PPARs through several different mechanisms. First, phosphorylation can alter PPAR's affinity for ligands even if the phosphorylation site is far from the ligand binding site through interdomain communications and conformational changes. Secondly, phosphorylation can modify PPAR's interactions with coactivators and corepressors. Phosphorylation-induced inhibition of transcriptional activity of PPARs provides a mechanism to switch off responses to ligand binding (Diradourian et al., 2004). PPAR phosphorylation could also modulate binding to PPRE. In addition, phosphorylation is a signal for ubiquitination and proteasomal catabolism of many proteins as well as a determinant of nuclear translocation with phosphorylation inhibiting translocation (Diradourian et al., 2004). However, it is important to remember that activities of PPARs are not only dependent on phosphorylation; they also require heterodimerization, cofactors, and ligands to elicit their effects, which further allude to their complex regulation.

PPARs in Salmonids: PPARs are widely studied in mammals, but little is known about fish PPARs. In brown trout, PPARα is highly expressed in white muscle, heart, and liver, while PPARβ predominates in testis, heart, liver, white muscle, and trunk kidney. PPARγ, however, was only quantified in the trunk kidney and liver (Batista-Pinto et al., 2009). PPARγ was first characterized in Atlantic salmon by Ruyter et al. (1997), with the full-length cDNA encoding for

PPARγ being reported by Andersen et al. (2000). There appears to be a more diverse expression of PPARγ in salmon where it is found not only in adipose tissue, but is also highly expressed in liver (Ruyter et al., 1997). Conversely, PPARγ was only quantifiable in the trunk kidney and very slightly in liver of brown trout (Batista-Pinto et al., 2009). Furthermore, Andersen et al. (2000) found three variants of PPARγ; two differing in 3'UTR length and the third has a truncated C-terminal. Batista-Pinto et al. (2009) determined that PPARβ was the dominant PPAR expressed in all tissues investigated (heart, liver, head kidney, trunk kidney, spleen, testis, blood, and white muscle). Furthermore, gender and stage of life cycle are known to influence expression levels of all PPARs in brown trout; estrogen appears to play an important role in differential expression of PPARs (Batista-Pinto et al., 2009). PPARα differences in gender were only during early vitellogenesis. There is also an increased expression of PPARβ in males pre-spawning. However, PPARγ expression was the same in male and female brown trout, increasing post-spawning (Batista-Pinto et al., 2009).

Target of Rapamycin (mTOR):

The TOR pathway is a central signaling pathway that plays a role in integrating energy-sensing pathways. Regulation of TOR provides a mechanism for cells to transition between anabolic and catabolic states in response to nutrient and energy availability (Laplante and Sabatini, 2011). TOR is a well-conserved serine/threonine kinase that regulates cell proliferation, growth, and metabolism (Hay and Sonenberg, 2004). TOR is a target of rapamycin, an anti-fungal macrolide produced by *Streptomyces hygroscopicus* isolated from soil (Vezina et al., 1975). Rapamycin is a highly specific inhibitor of TOR (Hay and Sonenberg, 2004). TOR was first identified through genetic screens of yeast, later mammalian TOR was characterized. The official name is now mechanistic TOR (mTOR) (Laplante and Sabatini, 2011).

Signaling Cascade: mTOR associates into two distinct protein complexes, TORC1 (target of rapamycin complex 1) and TORC2 (target of rapamycin complex 2; Figure 8). TORC1 integrates four major signals: growth factors, energy status, oxygen, and amino acids to promote cell growth and metabolism (Laplante and Sabatini, 2009). TORC2 is activated by growth factors and regulates cell survival, metabolism, and cytoskeletal organization (Laplante and

Sabatini, 2009). Both complexes act to alter gene transcription of a variety of metabolic pathways including protein synthesis, lipid synthesis, adipogenesis, mitochondrial proliferation, oxidative metabolism, stress resistance, apoptosis, and inflammation (Lapanate and Sabatini, 2009; Caron et al., 2010; Laplante and Sabatini, 2011). TORC1 promotes protein synthesis through phosphorylation of S6K1 and 4EBPs (Caron et al., 2010). Whereas, TORC1 activates lipid biosynthesis through a separate signaling pathway than that used to up regulate protein biosynthesis.

The mTOR-activated lipid synthesis begins with growth factors activating Akt, Erk, and Rsk through tyrosine-kinase receptors activating PI3K (Caron et al., 2010). The receptor acts as an $\alpha_2\beta_2$ tetramer (Voet and Voet, 2004). The α subunits contain the extracellular binding site; β subunits anchor the receptor in the cell membrane, and contain the tyrosine kinase domain that elicits enzymatic activity of the receptor. When insulin or another growth factor binds the α subunits, it activates the tyrosine kinase activity of the β subunit which auto-phosphorylates tyrosine residues of the β subunits (Voet and Voet, 2004). Activated tyrosine kinase then phosphorylates second messenger proteins such as insulin receptor substrate-1 (IRS-1). Once IRS-1 is phosphorylated it acts as a docking protein for other protein messengers that mediate actions of growth factors (Voet and Voet, 2004). One protein that is activated by its phosphorylation is phosphatidylinositol-3-kinase (PI3K). IRS-1 causes reactions that convert phosphatodylinosityl-4,5-diphosphate (PIP2) to become phosphatodylinosityl-3,4,5-triphosphate (PIP3) (Voet and Voet, 2004). This activates Akt. Akt stimulates the movement of GLUT4 transporters to the cell's membrane. It also activates protein phosphatase-1 (PP-1) which dephosphorylates proteins in nutrient utilization pathways regulated by phosphorylation (Voet and Voet, 2004). Another group of second messengers activated by tyrosine-kinase receptors are in the Cbl-P messenger system. This system facilitates the movement of GLUT4 transporters into the plasma membrane as well (Voet and Voet, 2004). Gene expression can also be altered by growth factors through the activation of the RAS/Mek pathway that activates a series of MAP kinase cascade reactions that controls gene expression of target genes (Voet and Voet, 2004). Overall, this signaling pathway results in increased glucose uptake and increased glycogen, lipid, and protein synthesis.

TORC1: Lipogenic gene transcription is primarily increased by downstream effects of Akt, Erk, and Rsk on TORC1. The mTOR protein is regulated by several proteins involved in the formation of TORC1. These proteins include PRAS40, mLST8, FKBP38, and deptor. TORC1 cannot elicit its effects if one of these proteins associates with the complex. Raptor, however, is a positive regulator of TORC1, activating it (Caron et al., 2010). Additionally, there are four phosphorylation sites on TOR that also regulate its actions. They are Ser1261, Ser2448, Ser2481, and Thr2446. Ser2481 is an autophosphorylation site. Ser2481 is the only site that has shown affects of TOR activity via phosphorylation (Caron et al., 2010). PIP3 activates Akt through phosphorylation; Akt, in turn, phosphorylates and inhibits PRAS40 allowing Raptor to activate TORC1. Akt also phosphorylates and inhibits TSC1/2 allowing for activation of TORC1 (Laplante and Sabatini, 2009). Additionally, Akt, Ras, and ERK phosphorylate TSC1/2 inhibiting its actions allowing for the activation of TORC1 (Caron et al., 2004). TORC1 is also sensitive to hypoxia through the action of REDD1 (regulated in development and DNA damage responses), a hypoxia-induced protein that inhibits the actions of TORC1. Another protein that alters TORC1 activity is MO25, which is activated during bouts of energy stress and inhibits the actions of TORC1 by activating TSC1/2 (Caron et al., 2010).

Gene expression profiling experiments have shown that over 5% of the transcriptome is differentially expressed in response to rapamycin-mediated mTOR inhibition (Caron et al., 2010). TORC1 has several downstream affects that alter lipid synthesis. First, it facilitates cleavage of SREBP1 through a mechanism that is not yet established (Laplante and Sabatini, 2009). When cleaved, SREBP1 translocates into the nucleus and induces the transcription of several lipogenic genes including *acc, fas, gpat,* and *scd1* (Voet and Voet, 2004). In addition, it appears that Akt increases the transcription of SREBP1 (Laplante and Sabatini, 2009). Another transcription factor affected by TORC1 signaling is PPARγ. There is evidence that PPARγ expression and activation is dependent on TORC1. C/EBPα appears to be under similar TORC1 regulation (Laplante and Sabatini, 2009). Interestingly, SREBP1 activation increases synthesis of PPARγ ligands promoting the transactivation of PPARγ as a transcription factor (Laplante and Sabatini, 2009). A third downstream effect of TORC1 involving lipid synthesis is its effects on Lipin1. Lipin1 is a phosphatase that facilitates conversion of phosphatidic acid to DAG. Lipin1 also acts as a transcriptional co-activator for PPARγ (Laplante and Sabatini, 2009). Lipin1 is

regulated through phosphorylation; however, it remains unclear how mTORC1 affects its activity. There is evidence that Lipin1 is phosphorylated in response to insulin and amino acids in a rapamycin-sensitive fashion, suggesting that mTOR signaling may directly regulate adipogenesis and lipogenesis through control of Lipin1 activity (Laplante and Sabatini, 2009).

TORC2: The second mTOR complex, TORC2, is involved in cell survival, metabolism, and proliferation, but its signaling pathways and role in lipid metabolism are less understood than TORC1. TORC2 is comprised of mTOR, Rictor, mSin1, mLST8, and Deptor (Caron et al., 2010). Deptor is an inhibitor of TORC2 while Rictor, mSin1, and mLST8 help facilitate complex formation (Caron et al., 2010). There is little known about the role of TORC2 in lipid metabolism. There has been some evidence in lower organisms that suggest TORC2 acts as a negative regulator of lipid deposition in Rictor null worms (Jones et al., 2009). It is unclear if this same association is present in mammals. However, there is evidence that TORC2 phosphorylates and activates Akt. Activation of Akt allows it to elicit its effects on TSC1/2 and TORC1 (Caron et al., 2010).

Pathway Integration with β-Oxidation: Most studies are focused on mTOR's roles in facilitating synthesis pathways such as protein, lipid, and amino acid synthesis. There is also an appreciation for mTOR's role in facilitating the switch between glucose, amino-acid, and fatty acid metabolism through mTOR's signaling relationship with AMPK (Tokunaga et al., 2004). However, a few studies have shown that mTOR has effects on energy breakdown pathways such as fatty acid β-oxidation as well. Both studies investigating mTOR's role in β-oxidation used rapamycin as an mTOR inhibitor to elucidate mTOR's role in β-oxidation (Brown et al., 2007; Sipula et al., 2006). Brown et al. (2007) used primary rat hepatocytes in culture and found that inhibition of mTOR by rapamycin increased β-oxidation of exogenous fatty acids by 46% at 18 hours and 100% at 48 hours. They went on to show esterification of exogenous fatty acids and *de novo* lipid synthesis were reduced by 40% and 60%, respectively (Brown et al., 2007). Rapamycin-inhibition of mTOR also decreased gene expression of *acc* and *gpat*. These findings further suggest mTOR not only plays an important role in energy sensing, but also plays a role in regulating energy production (Brown et al., 2007). Sipula et al. (2006) showed β-oxidation is also increased in L6 myotubes and *in vivo* in S6K1-deficient mice when mTOR is inhibited by

rapamycin. These authors saw significant increases in activities of CPT1 and CPT2 in culture with subsequent increases in their mRNA levels *in vivo* (Sipula et al., 2006). The mechanism by which mTOR elicits its effects on fatty acid β-oxidation remains unclear. Sipula et al. (2006) suggested that mTOR directly acts on key oxidative genes and proteins and causes a flux through the β-oxidation pathway. Brown et al. (2007), however, suggested that decreased expression of *acc* during rapamycin-inhibition of mTOR causes a decrease in its product, malonyl-CoA. Malonyl-CoA is the first intermediate in the fatty acid synthesis pathway and acts to inhibit CPT1. In the absence of malonyl-CoA, CPT1 is active. These authors suggest that it is the decreased ACC activity producing less malonyl-CoA that allows for CPT1 activity to increase; therefore, increasing the flux of fatty acids through β-oxidation (Brown et al., 2007). Both authors do, however, admit that further clarification of the mTOR pathway and its role in β-oxidation is necessary.

mTOR in Fish: The TOR signaling pathway in fish is less characterized than that of mammals, however the consensus has been that the mTOR signaling pathway is highly conserved among species through limited *in vitro* and *in vivo* studies (Plagnes-Juan et al., 2008; Seiliez et al., 2008; Lansard et al., 2009; Lansard et al., 2010; Seiliez et al., 2011). Most studies involving salmonids are focused on effects of insulin (Plagnes-Juan et al., 2008; Lansard et al., 2010) or feeding regimen (Lansard et al., 2009; Seiliez et al., 2011) on energy, mostly protein, metabolism. There was some assessment of the lipid synthesis pathway by investigating gene expression of *fas, srebp1,* and *cpt1*. However, one study focused on differences in lipid deposition between two divergent bred lines of rainbow trout (lean and fat) and the role mTOR signaling plays in developing those phenotypes (Skiba-Cassy et al., 2009).

Dietary studies determined that feeding a high protein diet activates the mTOR signaling pathway and shows a subsequent increase in *fas* and *srebp1*gene expression and a decreased *cpt1* mRNA content (Seiliez et al., 2011). Lansard et al. (2009) determined feeding a plant-based diet verses a fishmeal-based diet does not alter mTOR signaling, but there were significant increases in fatty acid synthesis genes with partial and full fishmeal and fish oil replacement. An *in vitro* study using rainbow trout primary hepatocytes determined amino acids alone did not activate the mTOR pathway; however, insulin activated the mTOR signaling pathway confirmed by an up-

regulation of lipogenic (*fas, acyl,*and *srebp1*) and glycolytic (*glucokinase, 6-phosphofructo-kinase, and pyruvate kinase*) genes (Lansard et al., 2010). Subsequently, another study tested effects of insulin injections on mTOR signaling *in vivo* in fasted rainbow trout and subjected primary hepatocytes to glucose and insulin stimulation (Plagnes-Juan et al., 2008). These authors determined that insulin is required for mTOR activation through the PI3-kinase/Akt pathway as observed in mammals. Glucose is required for the insulin-induced up-regulation of *fas* gene expression. They also reported a decreased expression of *cpt1*, but this response was only observed *in vivo* as *cpt1* was undetectable in the primary hepatocytes (Plagnes-Juan et al., 2008).

Skiba-Cassy et al. (2009) took a different approach to investigating the role mTOR plays in controlling lipid synthesis. These authors wanted to determine if divergent selection for high and low muscle fat altered nutrient utilization through changes in mTOR signaling of rainbow trout. They also fasted fish and subsequently measured changes in gene expression and mTOR activation during refeeding (Skiba-Cassy et al., 2009). Body weight or feed intake was not different between the two groups. All data further suggest insulin regulation of the mTOR signaling pathway is similar to that in mammals. Refeeding increased expression of lipogenic genes and *srebp1*. They also determined *mtor* was more abundant in the liver of the fat line fish. In addition, they found *cpt1* expression was low in the fat line fish compared to that of the lean line fish, suggesting a decreased ability for β-oxidation (Skiba-Cassy et al., 2009). Combining these findings and those of Corraze et al. (1999) who determined that *de novo* synthesized lipids are preferentially incorporated in muscle rather than adipose tissue, Skiba-Cassy et al. (2009) concluded genetic selection for increased muscle fat content results in over activation of the mTOR signaling pathway and increased expression of lipogenic genes. The aforementioned responses suggest there are metabolic differences in nutrient utilization between the lines resulting in different phenotypes (Skiba-Cassy et al., 2009).

Overview:

Many metabolic pathways, including those within fatty acid metabolism, are highly conserved across terrestrial and aquatic species. Unfortunately, there has been limited research on specific regulatory pathways in aquatic species. Investigating how fatty acid metabolism is regulated in fish will improve our understanding of lipid metabolism in aquatic species. Research

to characterize regulatory pathways and changes in fatty acid metabolism during various life stages would be beneficial in developing more standard protocols in fish culture and processing. Standard protocols for fish husbandry, similar to terrestrial food-animal species, will improve consistency of fillet quality. Fillet yields and quality are highly variable, making mechanical processing very difficult and wasteful. In general, investigations of lipid synthesis, mobilization, and oxidation throughout several life stages in fish will increase our understanding of fatty acid metabolism and how its regulation affects fillet quality in rainbow trout.

TABLES and FIGURES

Apoprotein	# of Residues	Molecular Mass (kD)	Function
AI	243	29	Activates LCAT
AII	77	17	Inhibits LCAT, activates hepatic lipase
B48	2152	241	Cholesterol clearance
B100	4536	513	Cholesterol clearance
CI	56	6.6	Activates LCAT
CII	79	8.9	Activates LPL
CIII	79	8.8	Inhibits LPL
D	169	19	Unknown
E	299	34	Cholesterol Clearance, tethers to HSPG

Table 1: Apoprotein Summary Table
This table summarizes the 9 apoproteins involved in lipid metabolism and their functions. LCAT—lecithin-cholestrol acyltransferase; LPL—lipoprotein lipase; HSPG-- heparin sulfate proteoglycan

	Chylomicron	VLDL	IDL	LDL	HDL
Density (g/cm3)	< 0.95	<1.006	1.006-1.019	1.019-1.063	1.063-1.210
Diameter (Å)	750-12,000	300-800	250-350	180-250	50-120
Mass (kD)	400,000	10,000-80,000	5,000-10,000	2,300	175-360
% Protein	1.5-2.5	5-10	15-20	20-25	40-55
% Phospholipid	7-9	15-20	22	15-20	20-35
% Free Cholesterol	1-3	5-10	8	7-10	3-4
% TAG	84-89	50-65	22	7-10	3-5
% Cholesterol Esters	36-5	10-15	30	35-40	12
Apoproteins	AI, AII, B48, CI, CII, CIII, E	B100, CI, CII, CIII, E	B100, CI, CII, CIII, E	B100	AI, AII, B48, CI, CII, CIII, D, E
Primary role in Transport	TAG and cholesterol from intestines to liver	TAG from liver to tissues	TAG to tissues	TAG to tissues	Cholesterol from tissues back to liver
Source of TAG	Diet	*De novo* synthesis	*De novo* synthesis	*De novo* synthesis	Little TAGs
Fed/Fasted State	Fed	Fed	Fed	Fed	N/A
Tissue Origin	Intestine	Liver	Blood Stream	Blood Stream	Liver
Synthesis	ApoB48 constitutively expressed	ApoB100 constitutively expressed	From VLDL	From IDL	ApoA1 constitutively expressed
Control of Synthesis	TAG available for packaging	TAG available for packaging	Rate of LPL hydrolysis of TAG from VLDL	Rate of LPL hydrolysis of TAG from VLDL and IDL	Rate of cholesterol esterification by LCAT
Uptake	Liver	N/A	Liver	Liver	Liver
Control of Uptake	Receptor mediated endocytosis by LDL-receptor like protein	N/A	Receptor mediated endocytosis by LDL-receptor	Receptor mediated endocytosis by LDL-receptor	Receptor mediated endocytosis by ApoA1 receptor or SR-B1 Receptor

Table 2: Lipoprotein Summary Table
This table summarizes properties and functions of lipoproteins.

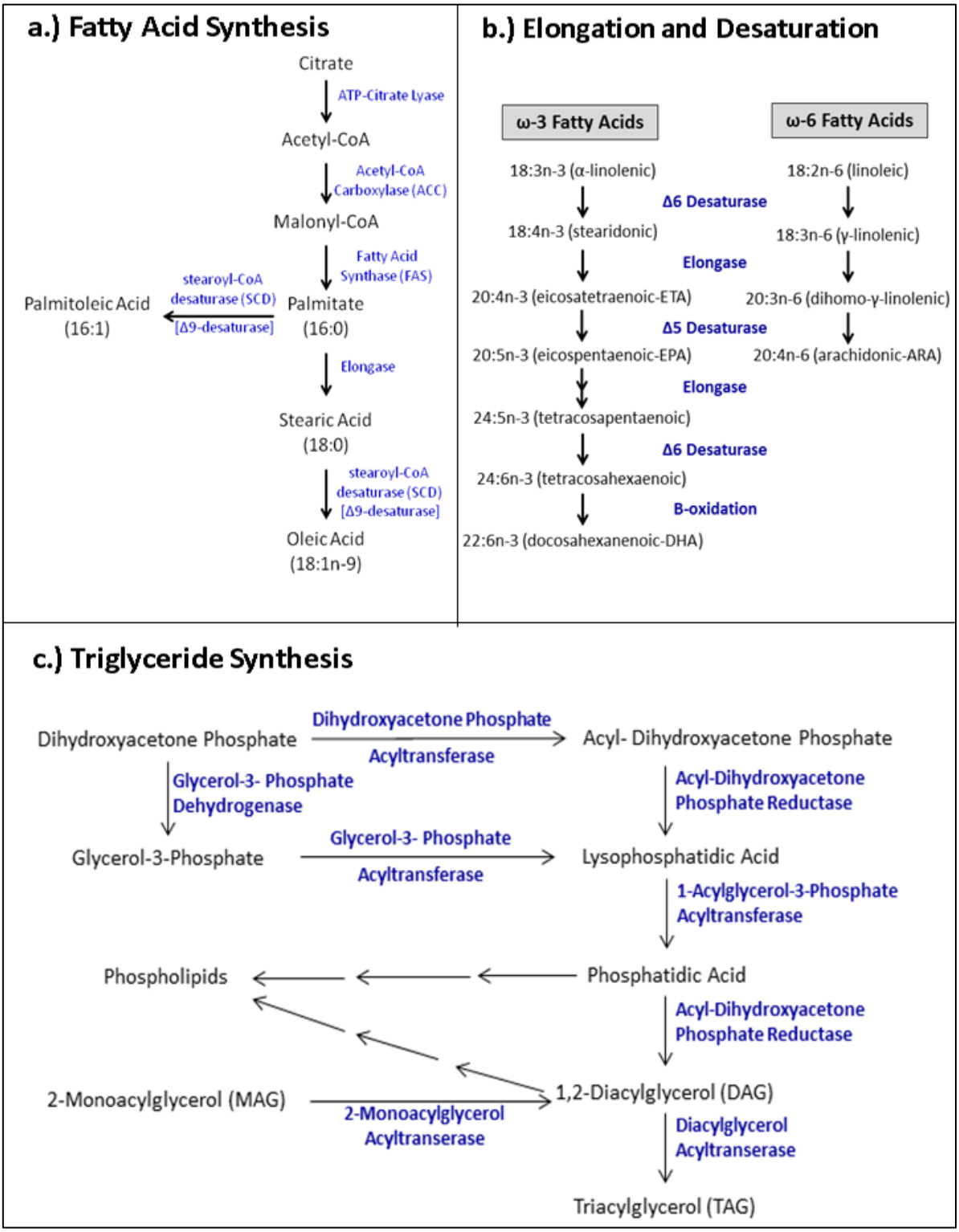

Figure 1: Fatty Acid and Triglyceride Synthesis Pathways
a.) Illustrates the fatty acid synthesis pathway; b.) Illustrates the omega-3 and omega-6 synthesis pathways from 18:2n-6 and 18:3n-3; c.) Illustrates the triglyceride synthesis pathway

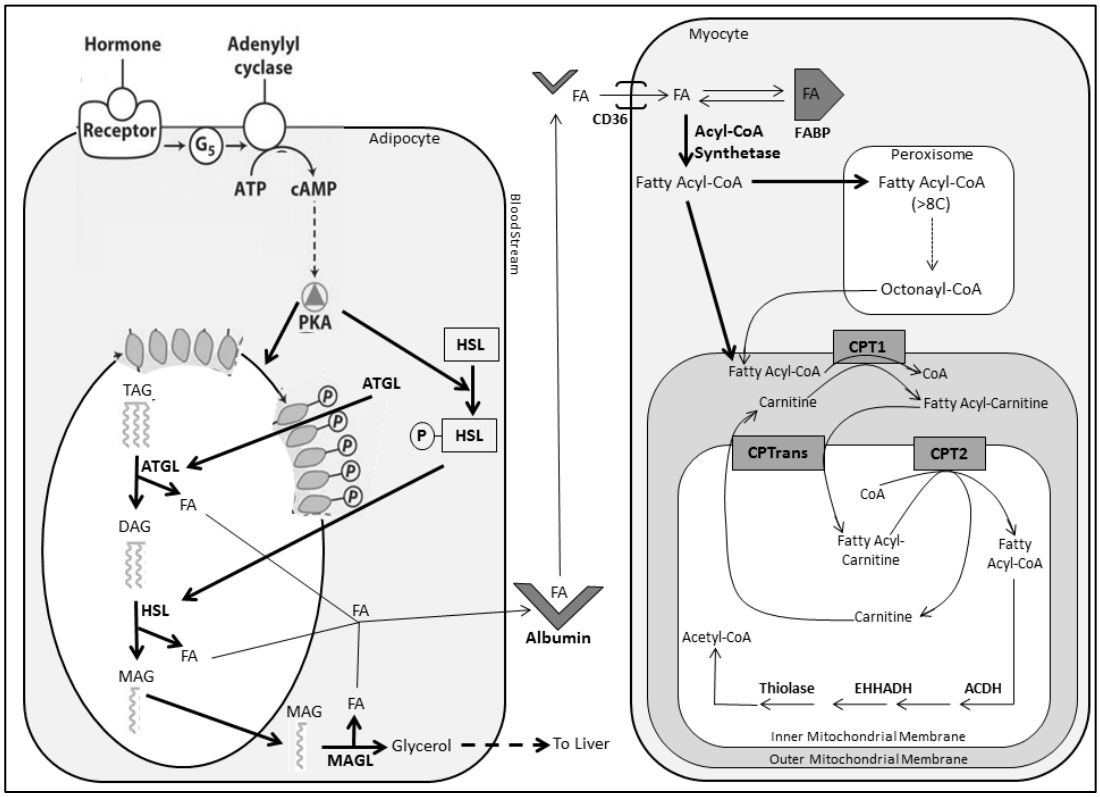

Figure 2: Lipolysis and β-Oxidation

Lipolysis: To mobilize TAGs and fatty acids from an adipocyte lipid droplet perilipin becomes phosphorylated to allow the translocation of lipases. ATGL hydrolyzes the fatty acid in the R3 position. HSL then hydrolyzes the fatty acid in the R1 position. The resulting MAG is released into the cytosol where MAGL removes the final fatty acid in the R2 position yielding the glycerol backbone. Glycerol is readily diffusible and goes to the liver to be converted to G3P by glycerol kinase. Free fatty acids are transported through the blood by albumin. Once albumin reaches a target cell, fatty acids are transported into the cell by fatty acid translocase/cluster of differentiation 36 (FAT/CD36), but must be bound to fatty acid binding proteins (FABP) or be metabolized immediately. ***Fatty Acid Oxidation: A***cyl-CoA synthetase forms a fatty acyl-CoA. The fatty acyl-CoA must then be transported into the mitochondria by carnitine palmitoyltransferase I (CPT1). Translocation of the fatty acyl-CoA is mediated by a protein carrier that transports acyl-carnitine into the mitochondria while transporting free carnitine out. In this process CPT1 transfers the fatty-acyl group to carnitine releasing CoA. Fatty-acyl carnitine is then transported into the mitochondria by CP translocase as it subsequently transfers free carnitine out. Fatty-acyl carnitine is then converted back to fatty acyl-CoA and free carnitine by CPT2 located on the inner mitochondrial membrane. Free carnitine is transported out of the mitochondria by CP translocase and the fatty acyl-CoA can enter the β-oxidation pathway. There are four steps that are repeated until the fatty acid is completely broken down into acetyl-CoA. These steps are carried out by acyl-CoA dehydrogenase (ACDH), enoyl-CoA hydratase, 3-L-hydroxyacyl-CoA dehydrogenase (EHHADH), β-ketoacyl-CoA thiolase These four steps are repeated until all of the carbons have been broken down to acetyl-CoA. Long-chained fatty acids undergo peroxisomal β-oxidation which is basically the same process, but it occurs in the peroxisome of the cell.

Enzyme	Reaction	Activated by	Insulin Action
Fatty Acid Synthesis			
Acetyl-CoA Carboxylase (ACC)	Acetyl-CoA ⟶ Malonyl-CoA	Dephosphorylation	+
Fatty Acid Synthase (FAS)	Malonyl-CoA ⟶ Palmitate	Dephosphorylation	+
Fatty Acid β-Oxidation			
Carnitinepalmitolytransferase (CPT1)	Fatty-Acyl CoA Transport	Malonyl-CoA	-
Lipolysis			
Perilipin	Lipases translocation	Phosphorylation	-
Hormone Sensitive Lipase (HSL)	DAG ⟶ MAG	Phosphorylation	-

Table 3: Insulin Actions on Fatty Acid Metabolism
This table summarizes insulin's effects of regulated enzymes within fatty acid metabolism pathway.

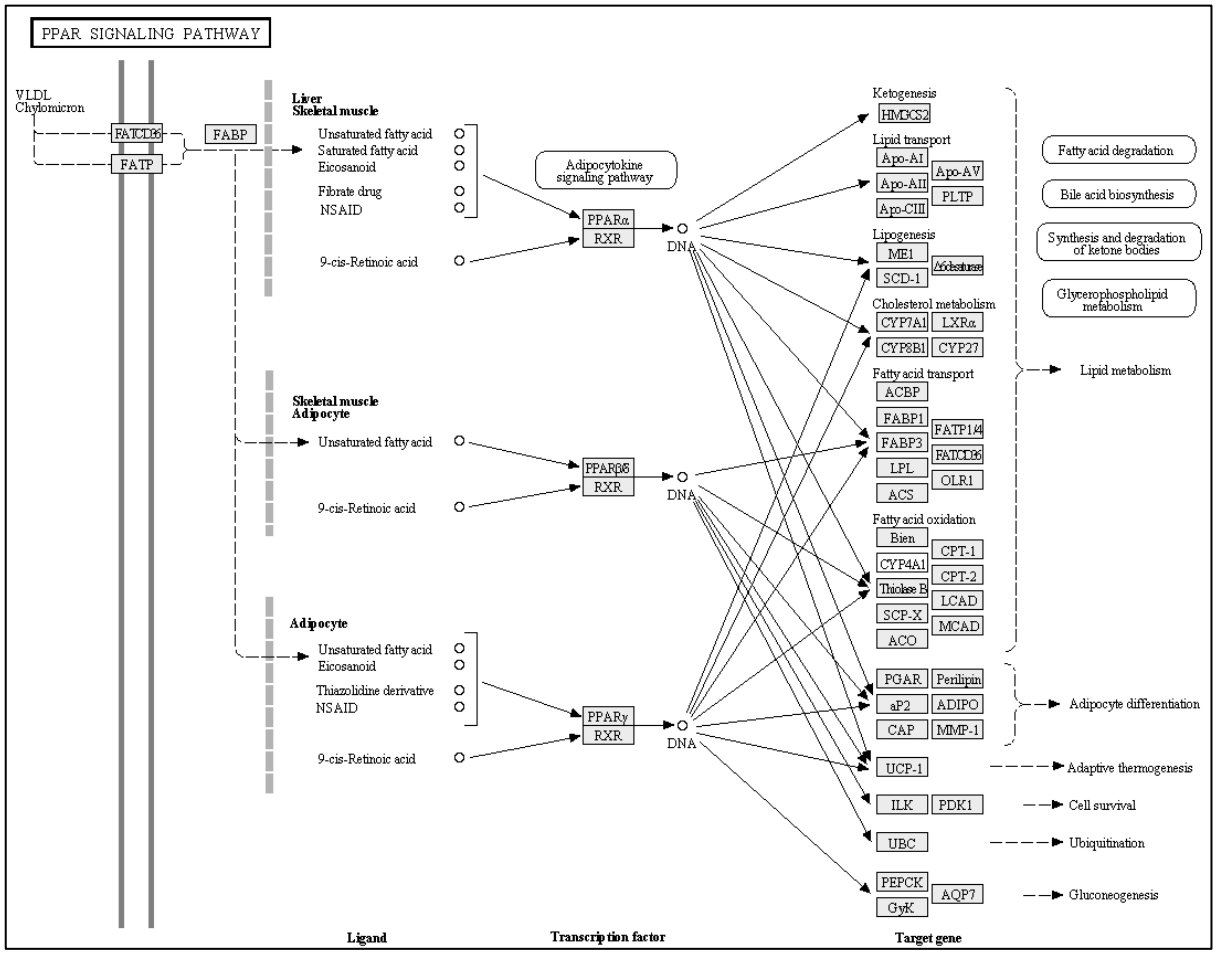

Figure 3: Perixosome Proliferator-Activated Receptor (PPAR) Signaling
This figure illustrates various effects of PPAR signaling on gene transcription (KEGG Pathways).

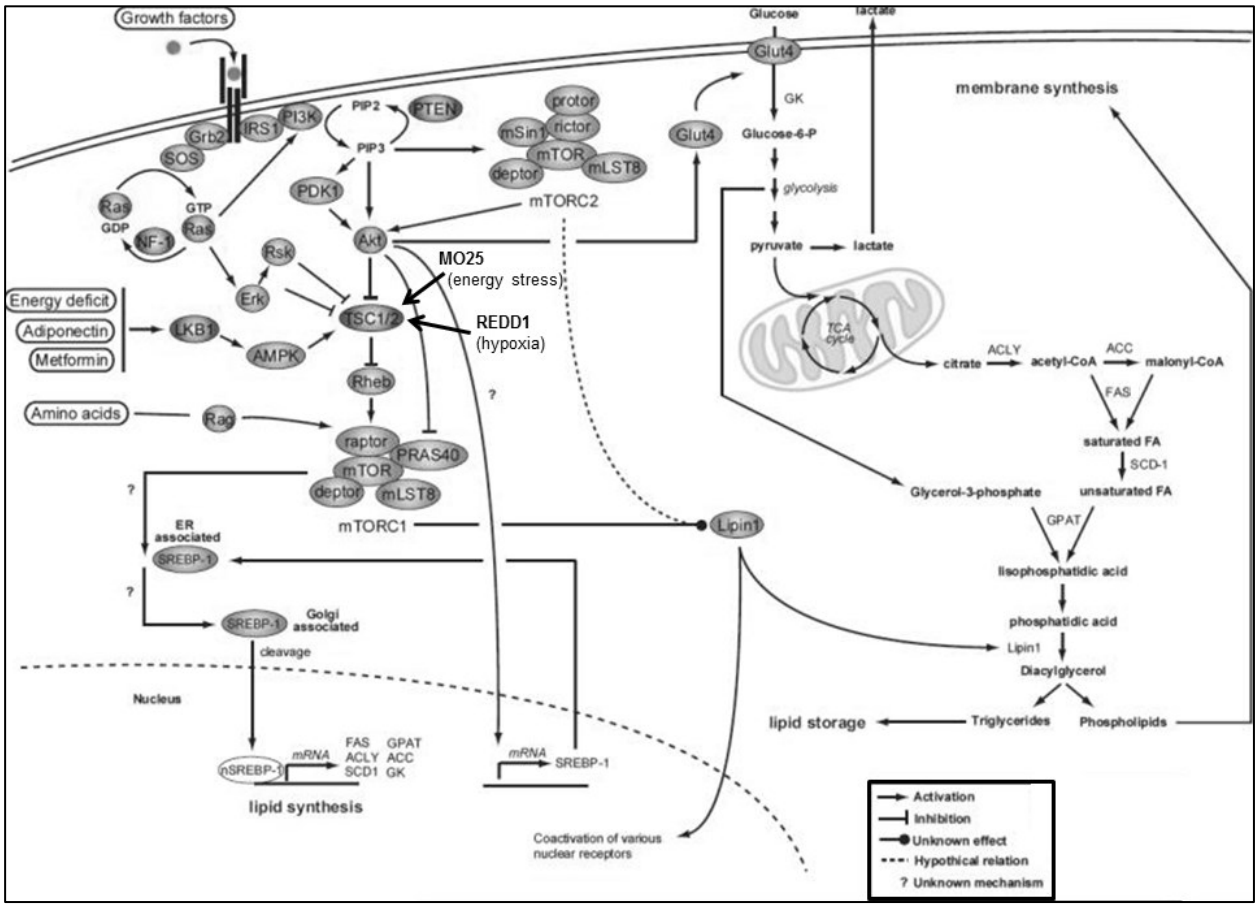

Figure 4: Mechanistic Target of Rapamycin (mTOR) Signaling Pathway
This figure illustrates the mTOR signaling cascade and its downstream effects on fatty acid metabolism (Laplante and Sabatini, 2009).

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CHAPTER 1

Title

Effects of feeding level and sexual maturation on fatty acid composition of energy stores in diploid and triploid rainbow trout (*Oncorhynchus mykiss*)

Authors

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Keywrods

salmonid, polyploidy, sexual maturation, fatty acids, energy stores

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ABSTRACT

Sexual maturation is an energy demanding, physiological process that alters growth efficiency and compromises muscle quality in many food-fish species. Lipid mobilization supplies energy required for this process. To study the effect of ration level on fatty acid composition, diploid (2N) rainbow trout, approaching ovulation, were fed at 0.25 and 0.50% of tank biomass/day and to apparent satiation (~0.75% of tank biomass/day). In addition, triploid (3N) female trout, which exhibit only minimal ovarian development, were fed at 0.50% of tank biomass/day. The primary objective of this study was to determine effects of ration level on fatty acid composition in different lipid compartments (muscle, visceral adipose tissue, liver, and gonad) during sexual maturation. Lower feeding levels produced smaller fish, but did not affect the onset of sexual maturation. Higher feeding levels resulted in fish muscle with higher relative amounts of saturated fatty acids (SFA), but monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were not affected by ration level. While ration level affected the fatty acid profile of each of the four tissues analyzed, the number of fatty acids affected was greatest in white muscle. An additional objective was to determine differences in the fatty acid composition of energy stores during maturation in female rainbow trout that were fed at a moderately restricted feeding level (0.50% of tank biomass/day). These differences were determined by comparing mature 2N to sterile 3N females of the same age. Diploid muscle contained higher amounts of PUFA (44.4±1.0%) than 3N muscle (39.7±0.8%). Saturated fatty acids were in the highest concentrations in muscle and visceral adipose tissue, and 2N liver contained more PUFAs and fewer MUFAs than 3N liver; however these values are relative values. In general, fatty acids affected by ration level were not the same as fatty acids affected by ploidy. Triploid fatty acid profiles did not mimic those of the satiation fed group; which was expected if 3N fish were simply consuming excess energy. Both 2N and 3N muscle fatty acid profiles were similar to that of the diet, except muscle had lower amounts of PUFA precursors (18:3n-3 and 20:5n-3) and higher relative amounts of their product (22:6n-3). Also, 2N muscle had higher 16:1 and 3N muscle had higher 16:0 compared to the diet. It is unclear if these differences are hormonally driven or if there are other physiological dissimilarities between 2N and 3N trout causing these differences. Overall, our data suggest that 2N and 3N fatty acid metabolism is regulated differently.

INTRODUCTION

Sexual maturation is a dominant physiological process that causes a shift from somatic growth to gonadal growth (Taranger et al., 2010). In many cultured fish species, including salmonids, gonadal development occurs at the expense of stored energy and nutrients, including lipids. During this time period, females cannot assimilate enough nutrients from the diet to support gonadal development (Nassour and Legar, 1989; Shearer, 1994; Jonsson et al., 1997; Kiessling et al., 2001; Memis and Gun, 2004; Salem et al., 2006; Gorgun and Akpinar, 2007; Aussanasuwannakul et al., 2011; Riberio et al., 2011; Aussanasuwannakul et al., 2012; and Manor et al., 2012). This repartitioning alters body composition, in general, and muscle lipid content, specifically. Depletion of intramuscular fat and protein catabolism in cultured rainbow trout results in a reduction in muscle quality; softer fillets with minimal fat are less desirable for food products (Rasmussen 2001; Salem et al., 2006; Salem et al., 2007; Cleveland et al., 2012), particularly in a species where a fillet with more oil is a standard of identity. During sexual maturation lipid is mobilized initially from visceral adipose tissue; although, in the long term, lipid will be mobilized from secondary storage sites such as muscle (Tocher 2003; Manor et al., 2012). In disagreement, Kiessling et al. (1991a) suggest that intramuscular fat acts as a short-term fat depot and is mobilized first. However, effects of sexual maturation on composition will likely depend on the size and composition of nutrient reserves, diet composition, and ration levels.

One method that can be used to avoid deterioration of muscle quality during sexual maturation is induction of triploidy. Triploid (3N) fish have three sets of chromosomes as opposed to two sets of chromosomes in normal diploid (2N) fish. The aquaculture industry induces triploidy in a variety of cultured species to cause sterility and prevent the onset of sexual maturation. In salmonids, such as rainbow trout, 3N females do not undergo sexual maturation and therefore do not develop large ovaries (Piferrer et al., 2009). Triploid females do not experience the shift from somatic growth to gonadal growth, thus preventing mobilization of lipids and deterioration of muscle quality. Triploid males do, however, undergo sexual maturation, but produce non-viable sperm (Piferrer et al., 2009). These characteristics make the

production of all female, 3N fish desirable within the aquaculture industry. Nevertheless, little is known about the differences in 2N and 3N fatty acid metabolism.

Since total lipid and specific fatty acid contents are important attributes of fillet quality, regulation of fatty acid profiles has received much attention. Considering variables that impact fatty acid composition, studies have addressed: 1) cultured versus wild fish, 2) seasonal variations, 3) altered diet composition, 4) fasting, and 5) basic physiology (Kiessling et al., 1989; Kiessling et al., 1991b; Kiessling et al., 2001; Regost et al., 2001; Memis and Gun, 2004; Menoyo et al., 2004; Haugen et al., 2006; Gorgun and Akpinar, 2007; Kandemir and Polat, 2007; Turchini and Francis, 2008). Of these variables, diet is the major contributor to muscle fatty acid composition. In general, white muscle saturated fat (SFA) and omega 6 (ω6) fatty acids are relatively stable while muscle monounsaturated (MUFA) and omega 3 (ω3) fatty acids exhibit greater sensitivity to changes in ration level. However, information on the responses of various lipid stores in fish to various ration levels is limited (Kiessling et al., 2001). In addition, little is known about differences in lipid metabolism between 2N and 3N rainbow trout. Our previous study, Manor et al. (2012), investigated fatty acid and proximate compositions of lipid stores in 2N and 3N rainbow trout on a high nutritional plane throughout sexual maturation and ovulation. We found that female rainbow trout on a high nutritional plane, with large visceral adipose tissue energy stores, did not mobilize lipid from muscle energy stores during sexual maturation. These findings are in contrast to studies using fish on lower nutritional planes (Kiessling et al., 1989; Kiessling et al., 1991a; Kiessling et al., 2001; Memis and Gun, 2004; Gorgun and Akpinar, 2007; Salem et al., 2007). Most research has focused on muscle fatty acid composition, with less emphasis on other lipid stores (ie. visceral adipose tissue). This follow-up study investigates effects of ration level on carcass characteristics and fatty acid composition of energy stores in female rainbow trout. Additionally, effects of sexual maturation on the fatty acid profiles were determined by comparing maturing 2N to sterile 3N, female rainbow trout on a moderately restricted feeding level (0.50% of tank biomass/day). Additional data from this study on growth, fillet quality, and indices of protein degradation are reported in Cleveland et al. (2012). The objective of this paper is to determine the effects of sexual maturation and ration level on fatty acid composition of four distinct tissues (white muscle, visceral fat, liver, and gonad) representing primary fat depots that are central to lipid metabolism, redistribution, and storage.

MATERIALS and METHODS

Experimental Design

Fish care and experimentation followed guidelines outlined by the US Department of Agriculture (USDA) and the National Center for Cool and Cold Water Aquaculture (NCCCWA; U.S. Department of Agriculture, Agricultural Research Service) Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals*. Diploid and triploid female rainbow trout from 4 families (family A, B, C, and D) were generated and maintained at the NCCCWA. At the fingerling stage (~50g) and for individual identification, fish were implanted with passive integrated transponders (PIT-tags; Avid Identification Systems Inc., Norco, CA, U.S.A.) in the dorsal musculature. Fish were confirmed 2N or 3N by flow cytometry (Allen, 1983; Hershberger and Hostuttler, 2007). Multiple families were used to ensure genetic diversity. Fish were maintained indoors, under simulated ambient photoperiod, and supplied with partially-recirculated and treated spring water throughout the study. Water temperatures ranged from 12.4^{o}C to 14.0^{o}C.

One month prior to onset of this study, fish were fed at 0.75% of tank biomass/day. Initial ration levels for 2N females were: 1) 0.50% of tank biomass/day, 2) 0.75% of tank biomass/day, and 3) apparent satiation, and 3N females were fed at 0.75% of tank biomass/day. Two, 1000L tanks were assigned to each of the four treatments, with a total of 7 fish per family per treatment. Families were split between two tanks, with the first tank containing 4 fish from families A and B, and 3 fish from families C and D. The second tank contained 3 fish from families A and B, and 4 fish each from families C and D. Therefore, each tank contained an equal number of fish (n=14). Two weeks into the 12 week study, it was calculated that fish fed to satiation were consuming feed equivalent to 0.80-0.90% of tank biomass/day. At this time, 2N feeding levels were adjusted to: 1) 0.25% tank biomass/day, 2) 0.50% of tank biomass/day, and 3) apparent satiation (~0.75% of tank biomass/day) for the remaining 10 weeks to increase potential differences between the satiation and the next-lowest feeding level. The 3N fish feeding level was also decreased from 0.75% tank biomass/day to 0.50% tank biomass/day. Triploid fish were only fed at 0.50% of tank biomass/day, a moderately restricted feeding level, because our previous study (Manor et al., 2012) examined 3N females fed to satiation. Moreover, the

moderately restricted feeding level employed in the 2N portion of this study was applied to 3N fish in order to test for the effect of ploidy. Although all fish were expected to be female, males were found in two families. In family C, 8 of the 28 fish were males, and in family D, 13 of the 28 fish were males. Since there were not enough females in family D to allow for sampling, this family was excluded from the study. Only data from female fish were included in the analysis of this study. This criterion resulted in 2 fish per family per tank per ration (48 total fish) sampled in January at 22 M of age.

Fish were fed Zeigler G, floating, 5.0mm (3/16") pelleted feed (42% protein, 16% fat, 2% fiber; Zeigler Brothers, Inc.; Gardners, PA, U.S.A.) dispensed by automatic feeders (Arvotec, Huutokoski, Finland) that adjust feed released daily based on the predicted mass of the fish in the tank. The fatty acid profile of the feed is provided in Table 1. Feeders dispensed feed in multiple feeding events between 7am and 2pm. Fish from each tank were weighed monthly to maintain the accuracy of the feeding regimen. Feeders for those tanks fed to satiation dispensed feed at 0.50% of tank biomass/day, followed by hand-feeding at the end of day to apparent satiation. Feeding procedures were modified one month after the start of the experiment to reduce the number of feeding events; these modifications reduced competition for available feed by increasing the amount of feed provided per feeding. This approach promotes a more even feed consumption among individuals, especially in those tanks assigned to the lower ration levels. To collect dispensed feed, buckets were placed under the feeders for tanks receiving 0.25 and 0.50% rations. Collected feed was then hand-fed to the fish at 8am the next day, with a second feeding at 2pm, if during the first feeding event the fish reached satiation before all the collected feed was dispensed.

Sample Collection

Fish were weighed in November at 20 M of age. November data are reported in Cleveland et al. (2012). In January fish were harvested using an overdose of tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, CA, U.S.A.) at 300 mg/L. Body weights and lengths were recorded along with standard gravimetric measurements as reported in Cleveland et al. (2012). Subsamples of dorsal muscle, liver, and visceral adipose tissue were immediately frozen in liquid nitrogen and stored at -80^{o}C for gene expression, proximate

composition, and fatty acid analyses. The dorsal muscle sample was taken from the epaxial muscle just caudal to the pectoral girdle and subcutaneous fat was removed. Fish were processed the following day at West Virginia University's Muscle Foods Laboratory (Morgantown, WV, U.S.A.). Boneless, skinless fillets were removed from each fish and weighed.

Proximate analyses

Epaxial muscle subsamples were frozen in liquid nitrogen, powdered using a Waring commercial grade blender (Model 51BL31; Waring Commercial; Torrington, CT, U.S.A.), and stored at -80°C until analysis. Liver, visceral adipose tissue, and gonad samples were minced at the time of analysis. Moisture and lipid analyses were completed using AOAC approved methods (AOAC, 2000). Moisture content was determined by weighing the sample before and after an 18h drying period at 110°C. Crude lipid content was determined indirectly using petroleum ether, Soxhlet extraction. Sample weight was recorded before and after extraction, and the difference was expressed as a percent of the original weight. Whole fillet proximate analysis was reported in Cleveland et al. (2012).

Fatty Acid Analysis

Total lipids were extracted from epaxial muscle, liver, visceral adipose tissue, and gonads, according to Bligh and Dyer (1959), using a chloroform-methanol mixture (2:1 v/v). A 0.05g sample of minced visceral adipose tissue was used for fatty acid analysis. Fatty acids were methylated using the method described by Fritshe and Johnston (1990). Nonadecanoic acid (19:0) was used as an internal standard. Fatty acid, methyl esters (FAMEs) were quantified using a Varian CP-3800 Gas Chromatograph (Varian Analytical Instruments; Walnut Creek, CA, U.S.A.) equipped with a flame ionization detector. A wall-coated, open-tubular fused silica capillary column (100m length, 0.25mm inside diameter; Varian Analytical Instruments) was used to separate FAMEs. The stationary phase was CP-Sil 88, and nitrogen was the carrier gas at a flow of 0.3mL/min. A 10 to 1 split ratio was applied for all samples. An oven temperature of 140^{o}C for 5 min, followed by a temperature ramp of 3^{o}C/min to 235^{o}C, was used; 235^{o}C was held for 15 min. The total separation time per sample was 68.5 min. Injector (11-77 injector, Varian Analytical Instruments) and detector (Flame Ionization Detector-FID, Varian Analytical Instruments) temperatures were maintained at 270^{o}C and 300^{o}C, respectively. Sample FAMEs

were identified based on comparison to retention times of standard FAMEs (SupelcoTM quantitative standard FAME 37; Sigma-Aldrich, St. Louis, MO, U.S.A.). Peak area counts were computed by an integrator using the Star GC workstation version 6 software (Varian Analytical Instruments). Fatty acids were reported as percent of total fatty acids.

Statistical analysis

Data were analyzed using analysis of variance to test for main effects of ration level and family with the general linear models procedure (PC-SAS Version 9.1; 2004). A 2x3x3x2 (fish x family x ration x tank) design, equaling 36 treatment combinations, was used to test ration main effects; accounting for mortalities and excluding males, 32 fish were sampled. A 2x3x2x2 (fish x family x ploidy x tank) design was used to test the main effects of ploidy, equaling 24 treatment combinations; accounting for mortalities and excluding males, 22 fish were sampled. Effects were considered significant at $P \leq 0.05$. There were no significant effects of fish or tank. Differences between 2N and 3N fish at the 0.50% ration level were detected with a t-test analysis. Data are presented as LSMeans ± SEM (standard error of the mean).

RESULTS

Growth

Family Effects: Because there were no family-by-ration interactions for growth responses (Table 2), the main effects of family and ration level were considered separately. The only growth parameter affected by family was gonadosomatic index (GSI; P=0.0485). Percent separable muscle and whole body weight (WBW) were not significantly affected by family.

Ration Effects: The highest rations levels produced the heaviest fish ($P<0.05$). Percent separable muscle was not affected ($P>0.05$) by ration level. Those fish at the highest feeding level, the satiation group, had the highest muscle, percent crude fat (Table 2) followed by the moderately restricted feeding level, 0.50% of tank biomass/day. Visceral adipose tissue, proximate composition (Table 2) followed the same pattern as muscle proximate composition;

visceral adipose of the satiation group contained the highest percent crude fat, and the 0.25% of tank biomass/day ration group contained the lowest percent crude fat. Moisture content, but not crude fat, was affected by ration in liver and 2N gonad tissues with the higher ration levels having lower moisture contents (Table 2).

Ploidy Effects: Ploidy was used to determine the effects of sexual maturation on growth and adipose tissue metabolism by comparing gravid (2N) and sterile (3N) female trout of the same age. There were no family-by-ploidy interactions for growth responses (Table 2). The 2N females had the largest WBW ($P \leq 0.05$) compared to 3N females. Fertile, 2N females also had a higher GSI ($P \leq 0.05$) than sterile, 3N females, as expected. Percent separable muscle was greater ($P \leq 0.05$) in 3N than in 2N females. The crude fat content of 3N muscle, liver, and visceral adipose tissue was higher than in 2N females (Table 2). The moisture content of 3N muscle, liver, and visceral adipose tissue was lower than equivalent 2N tissues.

Fatty Acid Content

Muscle—There were no significant differences in fatty acid content between 0.50% of tank biomass and satiation ration levels; whereas, 10 of 23 fatty acids differed between 0.25% and 0.50% of tank biomass ration levels (Table 3; $P \leq 0.05$). Overall, SFA and ω6 fatty acids were reduced, while ω3 and ω3:ω6 ratios were greater for the 0.25% tank biomass ration level compared to the 0.50% of tank biomass and satiation groups. In general, muscle was composed, primarily, of 22:6n-3 and 18:1n-9. In addition, 11 of 23 fatty acids differed between 2N and 3N fish fed the 0.50% ration, but not all affected fatty acids were the same, and values were not consistent with trends observed for the effect of ration. Some 3N fatty acid values were altered in the direction of the satiation fed fish and others in the direction of the 0.25% of tank biomass ration fed group. It is assumed that, if 3N females were simply consuming excess energy, their fatty acid profile would mimic that of the 2N satiation group. Overall, SFAs were lower and PUFAs were higher in 2N fish than the 3N fish fed at 0.50% of tank biomass. Variation observed in PUFAs is explained by changes in the ω6 fatty acids; 2N fish had more ω6 fatty acids in the muscle.

Liver—The satiation ration level resulted in the lowest 14:0 and 18:3n-6 concentrations, while the lowest ration level resulted in the lowest 20:1 and 20:2 concentrations. Eicosapentaenoic acid (EPA; 20:5n-3) was highest at the 0.25% of tank biomass/day feeding level and lowest at the 0.50% of tank biomass/day ration level. The liver was primarily comprised of 22:6n-3 and 16:0. Increased levels of 16:0 in the liver indicate fatty acid synthesis because this fatty acid is the end product of fatty acid synthase. There were no effects of ration level on any of the fatty acid categories (SFA, MUFA, PUFA, ω3, ω6). Ploidy had greater effects on liver fatty acids than ration level (Table 4). Overall, 2N livers contained more PUFA and ω6 fatty acids, while 3N livers contained more MUFAs.

Visceral Adipose Tissue—Only 20:1 was different ($P \leq 0.05$; Table 5) between fish fed to 0.50% of tank biomass and to satiation. Overall, there were no differences in SFAs, MUFAs, PUFAs, ω3 fatty acids, or ω6 fatty acids between the 0.50% of tank biomass and satiation ration levels. The most restricted feeding level resulted in the highest relative level of MUFAs and the lowest level of PUFAs and ω6 fatty acids. Visceral adipose tissue is comprised of MUFAs and PUFAs, with the primary fatty acid being 18:2n-6. Ploidy affected, primarily, the long chain, SFAs and MUFAs; 2N visceral adipose tissue had lower amounts of these fatty acids than 3N visceral adipose tissue. However, ω6 fatty acids, 18:2n-6 and 20:3n-6, were higher in 2N than in 3N visceral adipose tissue. Lower ($P \leq 0.05$) amounts of PUFAs and ω6 fatty acids along with a higher ω3:ω6 ratio were observed in 3N adipose tissue

Diploid Gonads—The effect of ration level on 2N gonads was analyzed because 3N females do not develop sufficient gonadal tissue for analysis. There were no differences between 0.50% tank biomass and satiation ration level. In addition, there were no effects of ration level on total SFAs, MUFAs, PUFAs, ω3 and ω6 fatty acids, or ω3:ω6 ratio (Table 6). Diploid gonads were mainly comprised of 16:0 and 22:6n-3. Interestingly, a significant family effect was observed in all but 9 of the 23 fatty acids measured suggesting that genetics has a larger effect on gonad fatty acid composition than ration level.

DISUCSSION

This study is a follow-up experiment to our previous work. Manor et al. (2012) examined maturing female 2N and 3N rainbow trout on a high plane of nutrition throughout sexual maturation from 16 M to 24 M of age. They focused on animal growth and fatty acid composition of muscle, liver, visceral adipose tissue, and gonads. Fish employed in that study did not mobilize muscle fat in response to sexual maturation because of the high plane of nutrition; we therefore wanted to investigate the energy stores during sexual maturation when fish are on a lower plane of nutrition. The primary objective of the current study was to determine the effects of ration level on fatty acid composition of different energy stores during sexual maturation. We have published the effects of ration level and triploidy on growth metrics and protein regulation in muscle from this same study (Cleveland et al., 2012). The current paper presents the effects of ration and triploidy on muscle, liver, visceral adipose tissue, and gonad proximate composition and fatty acid profiles in maturing female rainbow trout.

The lowest ration level, 0.25% of tank biomass/day, produced lower weight fish. Ration level did not affect the onset of sexual maturation, but reduced ration levels did produce mature females with smaller eggs (Cleveland et al., 2012). A secondary objective was to determine differences in the fatty acid composition of energy stores between 2N and 3N female rainbow trout under moderate nutrient restriction. Our previous study found no differences in growth or separable muscle between 2N and 3N females at 22 M (in January) when fish were on a high nutritional plane (Aussanasuwannakul et al., 2011; and Manor et al., 2012). However, in the current study, WBW and separable muscle were affected by ploidy. Diploid females were heavier and yielded lower percent separable muscle than 3N females fed at 0.50% of tank biomass/day. A higher WBW with less separable muscle is likely attributable to gonad development in 2N fish. A larger gonadal mass increased processing losses associated with the viscera. Additionally, some muscle atrophy may have occurred as a result of protein catabolism to support egg development.

Although triploidy results in a higher quality fillet than fillets from fish whose muscle is deteriorated by maturation, there is concern that differences in metabolism and energy uses will result in altered fillet composition in 3N females (Piferrer et al., 2009). Triploid epaxial muscle had higher amounts of total SFAs and lower amounts of PUFAs, but there was no difference in the ω3:ω6 ratio between 2N and 3N fish fed at the same rate (0.50% of tank biomass/day). In general, the fatty acid profile of 2N and 3N muscle was similar to that found in the diet. The exceptions were the PUFA precursors (18:3n-3 and 20:5n-3) being higher in the diet than in the muscle and their product 22:6n-3 (DHA) being higher in the muscle than in the feed. In addition, 3N muscle had higher amounts of 16:0 but similar amounts of 16:1 compared to the diet. Diploids, on the other hand, had similar amounts of 16:0 but higher amounts of 16:1 compared to the diet. These data suggest that sexual maturation in diploid females alters fatty acid deposition within muscle.

The impetus behind changes in fatty acid mobilization during female sexual maturation is ovarian development. Ration level affected relative amounts of several individual fatty acids, but did not affect total SFAs, MUFAs, or PUFAs in 2N gonads. Salze et al. (2005) determined that fatty acids impact egg quality, particularly the highly unsaturated fatty acids such as 20:5n-3, 22:6n-3, and 20:4n-6. They determined that 20:4n-6 was the most important unsaturated fatty acid affecting egg quality (Salze et al., 2005). Interestingly, 20:4n-6 and 22:6n-3 were not affected by ration level in our study, and 20:5n-3 was highest in 2N gonads at the most restricted ration level. This observation supports Tocher (2003) who reported that 20:5n-3 is preferentially mobilized from muscle and visceral adipose tissue and is deposited in 2N gonads during gonadogenesis. Potentially, greater mobilization of fat from muscle and visceral adipose tissue at the most restricted feeding level enabled more 20:5n-3 to move into the oocytes. Furthermore, the smaller volume of these oocytes, compared to oocytes from animals on 0.50% of tank biomass or satiation rations (Cleveland et al., 2012), may affect the fatty acid profile in favor of increasing concentrations of critical lipids because these measurements are relative values. Although only approximately 30% percent of the individual fatty acids measured in this study were significantly affected by ration, over 50% were affected by family. This observation suggests that fatty acid composition and egg quality can be altered to some degree by diet, but may be primarily determined by genetics. Only three rainbow trout families were represented in

this study, and this low number limits what we can definitively conclude about the relative significance of ration versus genetics on the fatty acid profile of the oocytes. Nevertheless, previous studies in fish suggest that maternal genetics significantly affect egg quality (Brooks et al., 1997).

The primary adipose tissue stores that support gonadogenesis in salmonids include visceral fat, dorsal fat, and intramuscular fat associated with red and white muscle (Kiessling et al., 1991). Adipose tissue can be separated from visceral and dorsal adipose tissue, while white epaxial muscle and red muscle contain the myofibrillar component and intramuscular adipose tissue; red muscle contains more lipid than white muscle (Kiessling, 1989). Previous studies indicate that lipid content in adipose tissue and white epaxial muscle decrease (Nassour and Legar, 1989; Shearer, 1994; Jonsson et al., 1997; Kiessling et al., 2001; Aussanasuwannakul et al., 2011; Riberio et al., 2011; Aussanasuwannakul et al., 2012; and Manor et al., 2012) as fish progress through sexual maturation. Whereas, dorsal adipose tissue and red muscle lipid content remains fairly stable across this period (Kiessling et al., 1989). Because visceral fat and intramuscular fat associated with epaxial muscle represent the primary energy stores with the most responsive fatty acid profiles, we chose to investigate effects of ration and maturation within these tissues.

Our data supports findings of Kiessling et al. (2001) that white muscle is the most responsive tissue to changes in ration level. Kiessling et al. (2001) suggested that SFAs and ω3 fatty acids are the most stable fatty acids while MUFAs and ω3 fatty acids are most responsive to changes in ration level. We, however, did not observe this trend in our study. Ration level altered SFAs, ω3 and ω6 fatty acids, and the ω3:ω6 ratio and did not affect the total MUFA or PUFA content of white muscle. For epaxial muscle from 2N females, higher ration levels increased SFA and ω6 fatty acids while the lower ration level resulted in more PUFAs and ω3 fatty acids. This effect of ration on epaxial muscle, crude lipid was not observed in immature rainbow trout (Kiessling et al., 1989); nonetheless, this effect in maturing 2N fish from the current study likely resulted from increased energy demands of sexual maturation and subsequent mobilization of epaxial muscle and fillet lipid stores (Cleveland et al., 2012). It is expected that as ration level increased, deposition of SFAs increased, thus reducing the relative percentage of ω3 fatty acids.

Our data indicate that feeding at 0.50% of tank biomass/day will yield 2N female rainbow trout with growth and epaxial muscle fatty acid composition similar to that of fish fed to apparent satiation. This finding is in agreement with previous data also indicating that fatty acid profiles of the epaxial muscle are not affected as ration levels approach satiation (Kiessling et al., 1989).

The other primary energy store is visceral adipose tissue. Several studies have shown that visceral fat is mobilized first to supply energy for gonadogenesis (Nassour and Leger, 1989; Jonsson et al., 1997; Manor et al., 2012). Fish will also mobilize intramuscular fat as a secondary energy source to support gonadogenesis when visceral reserves are low. Ration levels in this study required maturing fish to mobilize visceral and muscle fat. This effect of gonadogenesis is evidenced by lower muscle crude fat content and less visceral adipose tissue. Although we did not separate and quantify changes in visceral adipose tissue, a lower percent gastrointestinal tract (GtSI) of 2N compared to 3N females fed at the 0.50% of tank biomass/day indicates there was mobilization of visceral lipids shown through the reduction in this adipose tissue compartment as a proportion of the viscera (Cleveland et al., 2012). Differences in muscle fat content between 2N and 3N females were not observed in Manor et al. (2012) where fish had accumulated large amounts of fat in the various depots. In that study, mature 2N female rainbow trout had a GtSI of 7.5±1.5% and fillet crude fat content of 7.5±2.3%, and 3N females of the same age had a GtSI of 14.7±1.5% and fillet crude fat content of 11.7±2.3% (Aussanasuwannakul et al., 2011; Manor et al., 2012). To compare, 2N females in the current study had a GtSI of 3.97±0.24% and fillet crude fat content of 6.67±0.47% while 3N females had a GtSI of 8.08±0.23% and fillet crude fat content of 7.25±0.32% (Cleveland et al., 2012). The GtSI is used as a proxy to compare amounts of visceral adipose tissue by assuming little effect of ration or ploidy on the other organs of the gastrointestinal tract. The reduced muscle fat content and GtSI indicates that feeding at 0.50% tank biomass/day did not supply enough energy to prevent mobilization of endogenous lipid stores. Furthermore, GtSI and muscle fat content were not significantly improved with satiation feeding, suggesting that maximal levels of feed intake are still not sufficient to overcome the energy demands of reproduction in this study. This finding contradicts findings in Manor et al. (2012) which only had changes in visceral fat content with no effect of ploidy or sexual maturation on muscle fat content, albeit, Manor et al. (2012) had fish with much larger energy stores.

Effects of ration level on the fatty acid composition of visceral adipose tissue were not as dramatic as observed in the muscle, in agreement with Kiessling et al. (1991b) who determined that white muscle shows the largest changes in fatty acid composition with ration level when compared to other fat depots. Furthermore, 2N fish did not mobilize SFAs from visceral fat for energy during gonadogenesis as previously seen in Manor et al. (2012). Moreover, the current study supports Kiessling et al. (2001) in that muscle fat is used first as an energy store followed by visceral fat. Conversely, Manor et al. (2012) showed that visceral fat was the first energy store to be used to support gonadogenesis. These differences in findings could be a result of variances in the plane of nutrition and the accumulation of energy stores prior to the onset of sexual maturation.

In this study, and our previous study (Manor et al., 2012), SFAs were mobilized from 2N muscle and visceral adipose tissue in support of gonadogenesis. In the previous study (Manor et al., 2012), highly unsaturated fatty acids were also mobilized from visceral fat and muscle in support of gonadogenesis, but we did not observe this response in the current study. There is a selective catabolism of 20:5n-3, relative to 22:6n-3 in muscle, to produce energy for gonadogenesis which results in the selective transfer of 22:6n-3 to the eggs (Tocher, 2003). Kiessling et al. (2001) also showed an increased mobilization of 20:5n-3 during maturation, which was observed independent of ration level and only in visceral adipose tissue. However, in our study, 20:5n-3 and 22:6n-3 were not different between 2N and 3N fish in any of the tissues; this observation suggests that there was not preferential mobilization of either fatty acid to support gonadogenesis at the 0.50% ration level. In general, Kiessling et al. (2001) and Ribeiro et al. (2011) showed much lower levels of the PUFAs and much higher levels of MUFAs than our study for muscle and visceral adipose tissue, which could be attributed to variation in dietary lipid composition between the studies. Lower relative levels of PUFAs in 3N fish can be caused by increased amounts of SFAs stored as neutral triglycerides.

The liver is an important organ in fatty acid metabolism, but does not function as a significant fat store (Jonsson et al., 1997; Peragon et al., 2000). Few aspects of lipid metabolism are unique to the liver, but many are carried out predominantly by the liver. The liver is the primary site of fatty acid oxidation to produce acetoacetate for use by other tissues as energy. The liver also converts excess carbohydrates and proteins into fatty acids and triglycerides which are transported to adipose tissue depots for storage (Vemuri and Kelley, 2008). In addition, the liver is important in gonad development due to its role in vitellogenin synthesis (Memis and Gun, 2004). Ration level did not exert broad affects on the fatty acid profile of liver tissue indicating that the fatty acid profile of the liver is relatively unaffected by ration level during sexual maturation. Liver fatty acid composition was, however, significantly affected by ploidy. In general, 2N livers had less total MUFAs and more PUFAs and ω6 fatty acids than 3N livers. More frequent differences in liver fatty acid composition between 2N and 3N females indicate that sexual maturation (ploidy) had a stronger effect on liver fatty acid metabolism than differences in ration. These differences suggest that sexual maturation alters hepatic synthesis of specific fatty acids, mainly MUFAs and PUFAs. Alternatively, there can be inherent differences in fatty acid metabolism between 2N and 3N fish that contribute to differences in the fatty acid profile of other somatic tissues. Alterations in hepatic synthesis thus changes fatty acid deposition and mobilization in 2N and 3N white muscle and visceral adipose tissue.

CONCLUSION

Data from this study provides information about mobilization of lipid stores during moderate feed restriction at an important life stage. In general, restricting the ration level affects total SFAs, PUFAs, and ω3 and ω6 fatty acids in 2N muscle. It appears that fatty acids are mobilized to a greater extent from muscle of fish on more restricted diets. Our data indicate that feeding at 0.50% of tank biomass/day will allow optimal growth of 2N female rainbow trout, and feeding above that level will not affect growth or muscle and egg composition. Furthermore, our data suggests that fatty acid metabolism is differentially regulated in 2N and 3N females fed on a moderately restricted ration level.

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TABLES

Fatty Acid	Percent Fatty Acid (%)
14:0	7.1±0.8
16:0	19.8±0.5
16:1	7.1±0.1
18:0	3.7±0.2
18:1n-9	16.8±0.2
18:2n-6	21.5±0.1
18:3n-6	0.1±0.1
20:1	9.1±0.1
18:3n-3	3.0±0.1
20:4n-6	0.8±0.1
20:5n-3	6.5±1.0
22:6n-3	5.3±0.2

TABLE 1. FATTY ACID PROFILE OF THE DIET

Percent fatty acid of all measured fatty acids. All measurements were conducted in duplicate.

	Ration Level			Ploidy	P-values		
	0.25%	0.5%	Satiation	3N (0.5%)	Ration	Ploidy	Family
Growth Parameters							
WBW (g)	1584±71[a]	1888±89[b]	2015±76[b]	1331±43*	0.0018	<0.0001	0.1843
GSI (%)	13.7±0.7	12.6±0.9	13.9±0.8	0.04±0.40*	0.5598	<0.0001	0.0485
Separable Muscle (%)	40.6±2.1	43.9±2.6	44.5±2.2	51.2±3.2*	0.3979	0.0089	0.3779
Muscle Composition							
Moisture (%)	76.7±0.2[b]	75.3±0.2[a]	75.1±0.2[a]	74.3±0.3	<0.0001	0.0557	<0.0001
Crude Fat (%)	2.5±0.2[a]	3.0±0.3[b]	3.2±0.2[b]	4.2±0.3*	0.0549	0.0369	0.0557
Liver Composition							
Moisture (%)	78.1±0.3[b]	76.9±0.3[a]	76.8±0.3[a]	73.7±0.2*	0.0054	0.0054	0.7520
Crude Fat (%)	2.0±0.4	2.9±0.5	2.4±0.4	3.6±0.4*	0.4088	<0.0001	0.9937
Vis AT Composition							
Moisture (%)	16.2±0.9[b]	15.1±1.1[b]	11.8±1.0[a]	8.6±1.0*	0.0022	0.0022	0.2626
Crude Fat (%)	80.9±1.1[a]	82.4±1.3[a]	86.2±1.1[b]	90.3±1.1*	0.0016	0.0010	0.2331
2N Gonad Composition							
Moisture (%)	63.3±0.6[b]	60.0±0.8[a]	61.8±0.7[ab]	---	0.0114	---	0.0286
Crude Fat (%)	1.6±0.3	2.4±0.4	2.1±0.3	---	0.1998	---	0.6129
n	12	10	11	12			

TABLE 2: GROWTH AND PROXIMATE COMPOSITION—RATION EFFECTS

LSMean ± SEM for Whole Body Weight (WBW), Gonadosomatic Index (GSI), Percent Separable Muscle, and proximate composition of 2N female rainbow trout fed three ration levels (0.25% and 0.50% of tank biomass/day and satiation) and 3N female rainbow trout fed 0.50% of tank biomass/day. GSI was calculated by representing gonad weight as a present of WBW. Separable muscle was calculated by representing the boneless-skinless fillet weight as a percent of WBW. Moisture and crude fat contents of epaxial muscle, liver, visceral adipose tissue (Vis AT), and 2N gonad were measured using AOAC approved methods (AOAC, 2000). Superscripts (^{ab}) indicate ration main effects. Means with the same letters are not significantly difference ($p>0.05$). Asterisks (*) represent significant differences between 2N and 3N fish fed 0.5% tank biomass/day ($P\leq0.05$).

	Ration Effects					Ploidy Effects	
Fatty Acid	0.25 %	0.5 %	Satiation	Ration P-value	Family P-value	3N	Ploidy P-value
12:0	0.02±0.01	0.02±0.01	0.02±0.01	0.8344	0.1056	0.05±0.01*	0.0232
14:0	3.24±0.10[a]	3.62±0.12[b]	3.56±0.11[b]	0.0438	0.2511	4.17±0.10*	0.0040
14:1	0.06±0.01	0.04±0.01	0.05±0.01	0.3915	0.5138	0.08±0.01*	0.0025
15:0	0.26±0.01[a]	0.28±0.01[ab]	0.29±0.01[b]	0.0307	0.7859	0.29±0.01	0.3259
16:0	19.92±0.24	20.49±0.30	20.51±0.26	0.2016	0.6333	22.75±0.46*	0.0105
16:1	5.14±0.16	5.37±0.20	5.46±0.17	0.3737	0.0113	6.62±0.19*	0.0014
17:0	0.24±0.01[a]	0.26±0.01[b]	0.27±0.01[b]	0.0102	0.0662	0.25±0.01	0.0905
18:0	3.50±0.07[a]	3.76±0.08[b]	3.78±0.07[b]	0.0213	0.3935	4.06±0.08	0.0657
18:1n-9	16.26±0.30[a]	17.4±0.37[b]	17.24±0.31[b]	0.0376	0.0321	17.18±1.09	0.6641
18:2n-6	13.71±0.30[a]	14.81±0.37[b]	14.90±0.32[b]	0.0243	0.7166	13.44±0.29*	0.0212
20:0	0.13±0.004	0.14±0.01	0.14±0.005	0.4262	0.3309	0.15±0.03*	0.0414
18:3n-6	0.28±0.03	0.35±0.04	0.33±0.03	0.3196	0.0301	0.36±0.03	0.9268
20:1	5.12±0.21	4.79±0.26	4.96±0.22	0.6188	0.0959	5.00±0.26	0.4040
18:3n-3	1.49±0.04[a]	1.62±0.05[b]	1.73±0.05[b]	0.0037	0.3014	1.51±0.04	0.2295
20:2	1.38±0.11[a]	1.93±0.14[b]	1.80±0.12[b]	0.0136	0.3922	1.49±0.13*	0.0343
22:0	0.09±0.03	0.14±0.04	0.11±0.03	0.6174	0.5520	0.09±0.04	0.3529
20:3n-6	1.22±0.07	1.28±0.09	1.26±0.08	0.8677	<0.0001	1.05±0.06*	0.0354
22:1n-9	0.37±0.02	0.37±0.03	0.34±0.02	0.5421	<0.0001	0.45±0.03*	0.0316
20:3n-3	0.23±0.03	0.22±0.04	0.18±0.03	0.5889	0.3941	0.16±0.01*	0.0269
20:4n-6	1.65±0.07[b]	1.33±0.08[a]	1.40±0.07[a]	0.0124	0.0090	1.32±0.06	0.9320
20:5n-3	4.53±0.15[b]	3.51±0.20[a]	3.79±0.16[a]	0.0008	0.0488	3.17±0.10	0.0849
24:1	0.56±0.02	0.49±0.03	0.50±0.02	0.1497	0.2059	0.48±0.03	0.6864
22:6n-3	20.57±0.69[b]	17.76±0.86[a]	17.37±0.74[a]	0.0096	0.0623	15.87±0.65	0.1513
SFA	27.25±0.39[a]	28.74±0.45[b]	28.70±0.39[b]	0.0259	0.8976	31.8±0.6*	0.0117
MUFA	27.5±0.6	28.5±0.7	28.6±0.5	0.3743	0.0085	29.8±1.1	0.5673
PUFA	46.5±0.6	44.8±0.7	44.6±0.6	0.0689	0.0230	39.7±0.8*	0.0027
ω3	26.8±0.8[b]	23.1±1.0[a]	23.1±0.9[a]	0.0060	0.0504	20.7±0.7	0.0963
ω6	18.3±0.3[a]	19.7±0.4[b]	19.7±0.3[b]	0.0074	0.1943	17.7±0.4*	0.0057
ω3: ω6	1.47±0.06[b]	1.19±0.07[a]	1.18±0.06[a]	0.0046	0.0911	1.17±0.05	0.9606
n	12	10	11			12	

TABLE 3: EPAXIAL MUSCLE FATTY ACID COMPOSITION

Percent fatty acid of all measured fatty acids. LSMean±SEM for 23 individual fatty acids and the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (ω3), omega-6 fatty acids (ω6), and omega-3 to omega-6 ratio (ω3:ω6). Table shows means of 2N female rainbow trout fed three ration levels (0.25% and 0.50% of tank biomass/day and satiation). Superscripts indicate significant effects of ration. Table also shows the means of 2N and 3N female rainbow trout fed 0.50% of tank biomass/day. Asterisks (*) indicate ploidy main effects. Means without an asterisk are not significantly different ($p \leq 0.05$).

	Ration Effects					Ploidy Effects	
Fatty Acid	0.25	0.5	Satiation	Ration P-value	Family P-value	3N	Ploidy P-value
12:0	ND	ND	ND	ND	ND	ND	ND
14:0	2.08±0.13[b]	2.06±0.16[b]	1.63±0.13[a]	0.0494	0.2839	1.70±0.09	0.9012
14:1	ND	ND	ND	ND	ND	ND	ND
15:0	0.27±0.02	0.26±0.02	0.24±0.02	0.5524	0.2121	0.11±0.01*	<0.0001
16:0	20.06±1.19	20.66±1.47	21.75±1.26	0.6229	0.0960	21.51±0.78	0.1961
16:1	2.41±0.22	2.14±0.27	2.48±0.23	0.6261	0.2131	4.51±0.29*	0.0004
17:0	0.48±0.04	0.36±0.05	0.41±0.04	0.1668	0.0239	0.15±0.03*	0.0006
18:0	10.12±0.54	11.28±0.68	9.47±0.58	0.1543	0.6657	7.27±0.29*	<0.0001
18:1n-9	11.18±0.73	10.96±0.91	12.34±0.78	0.4450	0.6939	17.58±0.72*	<0.0001
18:2n-6	6.68±0.28	6.31±0.35	6.57±0.30	0.7110	0.3783	6.32±0.16	0.5638
20:0	0.08±0.02	0.10±0.02	0.09±0.02	0.7109	0.3390	0.24±0.02*	<0.0001
18:3n-6	0.21±0.02[b]	0.14±0.03[ab]	0.12±0.02[a]	0.0313	0.0199	0.25±0.02*	0.0080
20:1	3.75±0.26[a]	4.98±0.32[b]	3.54±0.27[a]	0.0069	0.0173	4.20±0.28	0.1208
18:3n-3	0.46±0.05	0.30±0.07	0.47±0.06	0.1191	0.0882	0.31±0.06	0.8688
20:2	2.68±0.27[a]	4.11±0.33[b]	3.36±0.29[ab]	0.0114	0.0006	2.63±0.26*	0.0121
22:0	ND	ND	ND	ND	ND	ND	ND
20:3n-6	3.29±0.42	4.14±0.52	2.81±0.45	0.1806	0.0983	2.92±0.28*	0.0153
22:1n-9	ND	ND	ND	ND	ND	ND	ND
20:3n-3	0.23±0.02	0.27±0.03	0.25±0.02	0.5166	0.1666	0.35±0.02*	0.0053
20:4n-6	5.23±0.64	4.68±0.80	4.74±0.68	0.8197	0.1126	3.15±0.23*	0.0003
20:5n-3	5.10±0.39[b]	3.35±0.48[a]	4.52±0.41[ab]	0.0358	0.6793	3.15±0.28	0.4224
24:1	1.44±0.13	1.54±0.16	1.89±0.14	0.0758	0.0100	2.12±0.16*	0.0355
22:6n-3	24.27±1.63	22.37±2.02	23.31±1.73	0.7635	0.4655	21.53±1.27	0.3905
SFA	33.08±1.72	34.72±2.14	33.60±1.83	0.8801	0.3185	30.98±1.10	0.1985
MUFA	18.78±1.09	19.61±1.35	20.25±1.16	0.6527	0.3714	28.41±0.93*	<0.0001
PUFA	48.14±2.78	45.66±2.83	46.15±2.42	0.7498	0.3918	40.61±1.59*	0.0310
ω3	30.05±1.96	26.28±2.43	28.56±2.08	0.4958	0.5164	25.34±1.52	0.4107
ω6	18.09±0.79	19.38±0.98	17.59±0.84	0.3920	0.2935	15.27±0.41*	<0.0001
ω3: ω6	1.71±0.13	1.39±0.16	1.63±0.14	0.3153	0.7513	1.66±0.10	0.2054
n	12	10	11			12	

TABLE 4: LIVER FATTY ACID COMPOSITION

Percent fatty acid of all measured fatty acids. LSMean±SEM for 23 individual fatty acids and the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (ω3), omega-6 fatty acids (ω6), and omega-3 to omega-6 ratio (ω3:ω6). Table shows means of 2N female rainbow trout fed three ration levels (0.25% and 0.50% of tank biomass/day and satiation). Superscripts indicate significant effects of ration. Table also shows the means of 2N and 3N female rainbow trout fed 0.50% of tank biomass/day. Asterisks (*) indicate ploidy main effects. Means without an asterisk are not significantly different ($p \leq 0.05$).

	Ration Effects					Ploidy Effects	
Fatty Acid	0.25	0.5	Satiation	Ration P-value	Family P-value	3N	Ploidy P-value
12:0	0.05±0.002	0.06±0.003	0.06±0.002	0.0763	0.3895	0.07±0.002*	0.0434
14:0	3.93±0.05	4.09±0.07	4.01±0.06	0.2287	0.0604	4.24±0.07	0.1238
14:1	0.11±0.004	0.12±0.005	0.12±0.004	0.5169	0.3601	0.15±0.004*	0.0027
15:0	0.46±0.01[a]	0.49±0.01[b]	0.50±0.01[b]	0.0026	0.2473	0.52±0.01*	0.0231
16:0	6.75±0.17[a]	7.40±0.22[b]	7.44±0.18[b]	0.0231	0.6610	9.48±0.20*	<0.0001
16:1	10.82±0.26[a]	11.61±0.32[ab]	11.75±0.27[b]	0.0468	0.9277	12.97±0.25*	0.0158
17:0	0.37±0.01	0.36±0.02	0.38±0.02	0.7729	0.8158	0.41±0.02	0.1269
18:0	2.32±0.05	2.18±0.06	2.24±0.05	0.2027	0.0355	2.75±0.05*	<0.0001
18:1n-9	17.93±0.23	17.72±0.28	17.81±0.24	0.8418	0.0645	17.84±0.16	0.6042
18:2n-6	30.19±0.43[a]	32.09±0.54[b]	31.27±0.46[ab]	0.0345	0.0005	26.86±0.43*	<0.0001
20:0	0.13±0.75	2.03±0.93	0.02±0.80	0.2244	0.2697	0.11±0.95	0.3380
18:3n-6	0.50±0.03	0.52±0.04	0.51±0.03	0.9268	0.2390	0.59±0.04	0.5086
20:1	12.01±1.01[c]	6.12±1.25[a]	8.73±1.07[b]	0.0044	0.3695	8.13±1.14	0.2239
18:3n-3	3.27±0.17	3.73±0.21	3.85±0.18	0.0662	0.0382	3.83±0.20	0.8308
20:2	1.92±0.11	1.93±0.14	1.76±0.12	0.5641	0.4821	1.70±0.11	0.2859
22:0	ND	ND	ND	ND	ND	ND	ND
20:3n-6	1.19±0.06	1.19±0.08	1.08±0.07	0.4743	<0.0001	0.90±0.06*	0.0194
22:1n-9	ND	ND	ND	ND	ND	ND	ND
20:3n-3	ND	ND	ND	ND	ND	ND	ND
20:4n-6	0.79±0.05	0.93±0.07	0.78±0.06	0.1960	0.8006	0.86±0.05	0.5784
20:5n-3	1.76±0.11	1.94±0.14	2.01±0.12	0.2980	0.2127	2.33±0.10	0.0947
24:1	0.38±0.08	0.43±0.11	0.30±0.09	0.6491	0.2739	0.31±0.11	0.6662
22:6n-3	5.05±0.36	5.05±0.44	5.39±0.38	0.7795	0.5970	5.94±0.41	0.3500
SFA	14.01±0.73	16.62±0.90	14.64±0.77	0.0983	0.4136	17.59±0.87	0.3685
MUFA	41.33±0.81[b]	36.00±1.00[a]	38.70±0.86[a]	0.0019	0.1512	39.40±0.97	0.0559
PUFA	44.66±0.69[a]	47.38±0.85[b]	46.65±0.73[ab]	0.0460	0.0103	43.01±0.67*	0.0011
ω3	10.08±0.57	10.72±0.70	11.25±0.60	0.3844	0.4215	12.10±0.65	0.3491
ω6	34.58±0.49[a]	36.66±0.61[b]	35.41±0.52[ab]	0.0499	0.0056	30.92±0.57*	<0.0001
ω3: ω6	0.29±0.02	0.30±0.02	0.32±0.02	0.5434	0.6410	0.39±0.02*	0.0227
n	12	10	11			12	

TABLE 5: VISCERAL ADIPOSE TISSUE FATTY ACID COMPOSITION

Percent fatty acid of all measured fatty acids. LSMean±SEM for 23 individual fatty acids and the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (ω3), omega-6 fatty acids (ω6), and omega-3 to omega-6 ratio (ω3:ω6). Table shows means of 2N female rainbow trout fed three ration levels (0.25% and 0.50% of tank biomass/day and satiation). Superscripts indicate significant effects of ration. Table also shows the means of 2N and 3N female rainbow trout fed 0.50% of tank biomass/day. Asterisks (*) indicate ploidy main effects. Means without an asterisk are not significantly different ($p \leq 0.05$).

	Ration Effects			P-values	
Fatty Acid	0.25	0.5	Satiation	Ration	Family
12:0	0.03±0.002[a]	0.04±0.003[ab]	0.04±0.002[b]	0.0239	0.0007
14:0	3.2±0.1	3.3±0.1	3.3±0.1	0.5268	0.0959
14:1	0.047±0.002[a]	0.050±0.003[ab]	0.059±0.003[b]	0.0147	0.0242
15:0	0.36±0.007	0.36±0.009	0.34±0.008	0.3480	0.9648
16:0	20.4±0.2	20.1±0.3	19.7±0.3	0.1221	0.0254
16:1	5.6±0.1	5.4±0.2	5.7±0.2	0.5683	0.0493
17:0	0.32±0.01[b]	0.30±0.01[ab]	0.23±0.01[a]	0.0131	0.1312
18:0	5.6±0.1	6.0±0.2	6.1±0.2	0.1109	0.0524
18:1n-9	0.26±0.04	0.34±0.06	0.22±0.05	0.2716	0.4167
18:2n-6	11.7±0.2	11.6±0.3	11.5±0.3	0.8494	0.0056
20:0	0.05±0.002	0.05±0.003	0.05±0.002	0.5819	0.5976
18:3n-6	0.47±0.02[b]	0.31±0.03[a]	0.26±0.03[a]	<0.0001	0.0011
20:1	2.9±0.07	3.1±0.09	3.1±0.07	0.0290	0.0645
18:3n-3	1.4±0.04	1.4±0.06	1.5±0.05	0.5884	0.1111
20:2	1.8±0.1	2.3±0.1	2.3±0.1	0.0009	0.0202
22:0	ND	ND	ND	ND	ND
20:3n-6	2.2±0.4	2.4±0.2	2.3±0.2	0.8088	<0.0001
22:1n-9	0.13±0.01	0.15±0.01	0.14±0.01	0.1622	0.1333
20:3n-3	0.20±0.01	0.22±0.01	0.21±0.01	0.3243	0.9785
20:4n-6	2.8±0.1	2.4±0.2	2.5±0.1	0.1255	0.0024
20:5n-3	4.2±0.1[b]	3.5±0.1[a]	3.6±0.1[a]	<0.0001	0.0110
24:1	0.33±0.01	0.34±0.02	0.35±0.01	0.5556	0.5616
22:6n-3	18.1±0.4	18.2±0.5	18.8±0.5	0.5126	0.0064
SFA	30.0±0.3	30.1±0.4	29.7±0.3	0.7836	0.0445
MUFA	27.0±0.3	27.5±0.4	27.2±0.4	0.6699	0.0002
PUFA	43.0±0.4	42.4±0.5	43.1±0.5	0.6229	0.1177
ω3	24.0±0.4	23.4±0.6	24.1±0.5	0.6173	0.0054
ω6	19.0±0.2	19.1±0.3	19.0±0.2	0.9800	0.0058
ω3: ω6	1.26±0.03	1.23±0.04	1.28±0.04	0.7108	0.0013
n	12	10	11		

TABLE 6: DIPLOID GONAD FATTY ACID COMPOSITION

Percent fatty acid of all measured fatty acids. LSMean ± SEM for 23 individual fatty acids and the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (ω3), omega-6 fatty acids (ω6), and omega-3 to omega-6 ratio (ω3:ω6). Table shows means of 2N female rainbow trout fed three ration levels (0.25% and 0.50% of tank biomass/day and satiation). Superscripts indicate significant effects of ration. Means with the same letters are not significantly different ($p \leq 0.05$).

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CHAPTER 2

Title

Effects of feeding level and sexual maturation on fatty acid metabolism gene expression in muscle, liver, and visceral adipose tissue of diploid and triploid rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

In many cultured fish species, such as salmonids, gonadal development occurs at the expense of stored energy and nutrients, including lipids. However, mechanisms regulating nutrient repartitioning during sexual maturation are not well understood. This study investigated effects of ration level and sexual maturation on expression of 35 genes involved in fatty acid metabolism, including genes within fatty acid synthesis, β-oxidation, and cofactors of the mTOR and PPAR signaling pathways. Gene expression profiles were assessed in liver, white muscle, and visceral adipose tissue of sexually maturing, diploid (2N) female rainbow trout fed at 0.25 and 0.50% of tank biomass/day and to apparent satiation. Additionally, sterile triploid (3N) female trout were fed at 0.50% of tank biomass/day for comparison to 2N females fed at the same ration level. Gene expression was affected by ration level only in white muscle; *erk* and *acat2* were increased in fish fed higher rations. On the other hand, sexual maturation affected gene expression across all three tissue types. Data indicate 2N fish have increased expression of β-oxidation genes within white muscle and within visceral adipose tissue. These findings support enhanced fatty acid mobilization within these tissues during sexual maturation. Increased expression of fatty acid synthesis genes in 3N female liver is associated with increased expression of mTOR cofactors and *pparγ*, which reflects continued deposition of lipids in these fish. Furthermore, increased expression of genes involved in β-oxidation pathways across ration levels in 2N females suggests that maturation-associated hormonal signals, such as estrogen, may regulate these effects.

INTRODUCTION

Sexual maturation is a dominant physiological process that causes a shift from somatic growth to gonadal growth (Taranger et al., 2010). In many cultured fish species, including salmonids, gonadal development occurs at the expense of stored energy and nutrients, including lipids. During this time period, female rainbow trout develop ovaries that account for over 20% of total body weight prior to ovulation (Tyler et al., 1990). Maturing females typically cannot

assimilate enough nutrients from the diet to support gonadal development and, therefore, must mobilize energy reserves to support the increased energy demand (Nassour and Legar, 1989; Shearer, 1994; Jonsson et al., 1997; Kiessling et al., 2001; Memis and Gun, 2004; Salem et al., 2006; Salem et al., 2007; Gorgun and Akpinar, 2007; Aussanasuwannakul et al., 2011; Riberio et al., 2011; Aussanasuwannakul et al., 2012; and Manor et al., 2012). Lipids are mobilized from visceral adipose tissue and muscle stores during maturation (Nassour and Legar, 1989; Shearer, 1994; Jonsson et al., 1997; Aussanasuwannakul et al., 2011; Manor et al., 2012). However, effects of sexual maturation on body composition likely depend on size and composition of nutrient reserves, diet composition, and ration levels. Even though nutritional plane during maturation affects egg size, it does not affect the proximate composition or fatty acid content of eggs (Ridelman et al., 1984; Knox et al., 1988; Washburn et al., 1990; Cleveland et al., 2012; Manor et al., 2014). The lack of changes in gonadal development in response to changes in dietary nutrient availability indicates the importance of endogenous nutrient reserves during sexual maturation in female rainbow trout (Nassour and Legar, 1989).

Although nutrient repartitioning is important to sexual maturation in female fish, little is understood about regulation of nutrient mobilization during this time period. Two pathways, known to regulate nutrient metabolism in mammals, are the mechanistic target of rapamycin (mTOR) and peroxisome proliferator activated receptors (PPAR) pathways (Laplante and Sabatini, 2011; Poulsen et al., 2012). Both pathways respond to nutrient availability and alter target gene expression of key enzymes involved in fatty acid metabolism. Nonetheless, both pathways are not specific to controlling lipid metabolism; they are also active in a variety of other processes such as inflammation, immune function, apoptosis, protein metabolism, and stress resistance (Laplante and Sabatini, 2011; Poulsen et al., 2012).

The mTOR pathway is a central signaling cascade that plays a role in integrating energy-sensing pathways. Regulation of mTOR provides a mechanism for cells to transition between anabolic and catabolic states in response to nutrient and energy availability (Laplante and Sabatini, 2011). The mTOR signaling pathway in fish is less characterized than that of mammals; however, through limited *in vitro* and *in vivo* studies, the consensus has been that the mTOR signaling pathway is highly conserved among species (Plagnes-Juan et al., 2008; Seiliez et al.,

2008; Lansard et al., 2009; Lansard et al., 2010; Seiliez et al., 2011). There are two main paths mTOR can act through; the assembly of mTOR Complex 1 (TORC1) and mTOR Complex 2 (TORC2). TORC1 elicits its effects on lipid metabolism by increasing the expression of genes involved in fatty acid synthesis (Laplante and Sabatini 2009; Caron, 2010). Whereas, TORC2 is less characterized, it is believed to play a role in regulating the transcription of genes involved in fatty acid β-oxidation (Jones et al., 2009). However, most studies involving mTOR in salmonids are focused on effects of insulin (Plagnes-Juan et al., 2008; Lansard et al., 2010) or feeding regimen (Lansard et al., 2009; Seiliez et al., 2011) on energy, mostly protein, metabolism. There has been some assessment of the fatty acid synthesis pathway by investigating gene expression of *fas, srebp1,* and *cpt1* (Lansard et al., 2009; Seiliez et al., 2011). However, one study focused on differences in lipid deposition between two divergently bred lines of rainbow trout (lean and fat) and the role mTOR signaling plays in developing those phenotypes (Skiba-Cassy et al., 2009).

The PPAR signaling pathway is known to respond to lipids and elicit transcriptional changes on genes involved in lipid metabolism in mammals. PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors (Poulsen et al., 2012). All three isoforms of PPAR must form a heterodimer with retinoid x receptor (rxr) in order to elicit their effects on gene transcription. Gender and stage of life cycle influence expression levels of all the PPARs in brown trout; estrogen appears to play an important role in differential expression of PPARs (Batista-Pinto et al., 2009). Activation of PPARγ specifically leads to increased TAG accumulation in muscle and liver (Pouslen et al., 2012). PPARγ is the most abundant of the PPARs, occurring in adipose tissue at levels 30-fold higher than levels found in other mammalian tissues (Varga et al., 2011). PPARγ affects transcription rates of a variety of lipogenic target genes such as *fabp, cd36, lpl, leptin, acc, fas, and scd1* (Lee and Hossner, 2002). Additionally, PPARα and PPARβ are responsible for regulating fatty acid β-oxidation (Varga et al., 2011).

This study is part of a series of publications examining effects of maturation and ration level on indices of protein degradation, fillet quality, body composition, and fatty acid content of energy stores in female diploid (fertile; 2N) and triploid (sterile; 3N) rainbow trout (Manor et al.,

2014; Cleveland et al., 2012). As previously reported, ration levels employed in this study did not negatively impact maturation or fatty acid body composition. There was, however, an up-regulation of gene expression within proteolytic pathways during sexual maturation that was also dependent on ration level. In addition, ration levels altered nutrient retention efficiencies and egg size. Conclusions among these studies were that the 0.50% ration level is an optimal feeding strategy for fish during the breeding cycle to increase efficiency and profitability. The purpose of this current report is to assess differences in lipogenic gene expression in response to varying ration levels and sexual maturation by comparing maturing 2N females to sterile 3N females of the same age. Understanding how genes within pathways related to lipid metabolism are regulated will indicate mechanisms responsible for nutrient repartitioning during sexual maturation. Furthermore, identifying critical genes and pathways associated with phenotypic traits will enhance our knowledge of how management strategies or feeding practices can regulate these mechanisms for more efficient food-fish production.

MATERIALS and METHODS

Experimental Design

Fish care and experimentation followed guidelines outlined by the US Department of Agriculture (USDA) and the National Center for Cool and Cold Water Aquaculture (NCCCWA; USDA—Agricultural Research Service; Kearneysville, WV, U.S.A.) Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals*. Experimental design was reported in detail by Cleveland et al. (2012) and Manor et al. (2013). Briefly, 2N females were fed at 1) 0.25% tank biomass/day, 2) 0.50% of tank biomass/day, and 3) apparent satiation (~0.75% of tank biomass/day) for 10 weeks. The 3N fish were fed at 0.50% tank biomass/day. Two, 1000L tanks were assigned to each of the four treatments, with a total of 7 fish per family (3) per treatment. Fish were fed Zeigler G, floating, 5.0mm (3/16”) pelleted feed (42% protein, 16% fat, 2% fiber; Zeigler Brothers, Inc.; Gardners, PA, U.S.A.) dispensed by automatic feeders (Arvotec; Huutokoski, Joroinen, Finland) that adjust feed released daily based on the predicted mass of the fish in the

tank. Feeders for those tanks fed to satiation dispensed feed at 0.50% of tank biomass/day, followed by hand-feeding at the end of day to apparent satiation.

Sample Collection

Fish were weighed and harvested in November at 20 M of age and in January at 22 M of age using an overdose of tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, CA, U.S.A.) at 300mg/L. Body weights and lengths were recorded along with standard gravimetric measurements as reported in Cleveland et al. (2012). Subsamples of dorsal muscle, liver, and visceral adipose tissue were immediately frozen in liquid nitrogen and stored at -80^{o}C for gene expression, proximate composition, and fatty acid analyses. Proximate and fatty acid compositions of liver, white muscle, gonad, and visceral adipose tissue are reported in Manor et al., 2014. Fish were processed the following day at West Virginia University's Muscle Foods Laboratory (Morgantown, WV, U.S.A.). Boneless, skinless fillets were removed from each fish, weighed, and reported in Cleveland et al. (2012).

Gene Expression Analysis

Multiplex Analysis

The GenomeLab GeXP genetic analysis system (Beckman Coulter Inc.; Pasadena, CA, U.S.A.) was used to simultaneously analyze expression of thirty-nine genes in liver, white muscle, or visceral adipose tissue. Within the multiplex, thirty-five genes were associated with fatty acid metabolic pathways and four served as potential reference genes. Primers were designed using eXpress Designer software (Beckman Counter Inc.; Pasadena, CA, U.S.A.) and primer sequences were compared against other rainbow trout gene sequences using the BLAST function within the NCBI database to reduce unintended sequence amplification. The size of each amplicon was confirmed with its expected length. No undetermined peaks interfered with amplification of the intended multiplex. Optimization of the multiplex, standard curve, reverse transcriptase (RT) and PCR reactions, and capillary electrophoresis were performed as recommended by the manufacturer (GeXP Chemistry protocol A29143AC; February, 2009) with reagents provided in the GeXP Start Kit (Beckman Coulter Inc.; Pasadena, CA, U.S.A.). GenBank accession numbers, database reference numbers, and references for sequences used to generate multiplex primers, the associated regulatory pathways, roles in lipid metabolism, and R^2

values for the RNA standard curve (0.2 ng /µL – 100 ng/µL), are shown in Appendix 4, Table 6. Primer sequences that include universal tags are provided in Appendix 3, Table 5.

Areas for each peak within the multiplex were exported to eXpress Profiler software (Beckman Coulter, Inc.; Pasadena, CA, U.S.A.) for analysis and normalization to the internal kanamycin control. Concentrations were interpolated from the standard curves for each gene of interest. Data were normalized to the highest expressing sample for input into GeNorm software to determine which reference genes were most stable. The most stable reference genes were *β-actin*, *rplp2*, and *ef1a* for all three tissues. M-values for these three genes and for all three tissues were below 0.5; therefore, their geometric mean was used to generate a normalization factor for each sample. Thus, the normalized expression of each gene transcript is reported as the quantity relative to the geometric mean of the selected reference genes.

RNA Isolation

To isolate RNA, 50 – 100 mg of tissue was homogenized in 1 mL TRIzol (Invitrogen; Carlsbad, CA, U.S.A.), per manufacturer's suggested protocol, using a 5 mm steel bead and a multi-tube shaker. The RNA pellet was washed with 75% ethanol and resuspended in nuclease-free water. RNA quality and quantity was determined by measuring absorbance at 260 nm and 280 nm.

Multiplex PCR

The multiplex RT reactions were optimized for each tissue as recommended by the manufacturer. Liver RT included 1.25 µL of 100 ng DNase-treated RNA in a 10 µL RT reaction that included 2 µL 5X RT buffer, 1 µL gene-specific reverse primer mix, 0.5 µL RT, and 1.25 µL kanamycin RNA (internal control, 1:2 dilution). White muscle RT reaction mixtures included 2.5 µL of 100 ng DNase treated RNA in a 10 µL RT reaction that included 2 µL 5X RT buffer, 1 µL gene-specific reverse primer mix, 0.5 µL RT, and 1.25 µL kanamycin RNA (internal control, stock). Visceral adipose tissue RT included 2.5 µL of 100 ng DNase treated RNA in a 10 µL RT reaction that included 2 µL 5X RT buffer, 1 µL gene-specific reverse primer mix, 0.5 µL RT, and 1.25 µL kanamycin RNA (internal control, stock). The RT was incubated according to kit instructions (48^{o}C for 1min, 42^{o}C for 60min, 95^{o}C for 5min, and 4^{o}C hold). An aliquot (4.65 µL)

of the resultant cDNA was used in PCR reactions for all three tissues that included 2 µL of 25 mM $MgCl_2$, 2 µL of 5X PCR buffer, 1 µL forward primer mix, and 0.35 µL DNA Taq polymerase. The PCR was incubated according to kit instructions (95°C for 10min, 95°C for 30 sec, 55°C for 30sec, 70°C for 1min, repeat steps 2-4 for an additional 34 cycles (35 cycles total), and 4°C hold). 1 µL of PCR products was combined with 38.5 µl sample loading solution and 0.5 µL size standard 400. The PCR products were separated by capillary electrophoresis in the GeXP Genetic Analysis System using a modified Frag-3 protocol with a separation voltage of 6.0 kV for 45 min.

Statistical analysis

Data were analyzed using analysis of variance to test for main effects of ration level, month, and family using PC-SAS (Version 9.1; Cary, NC, U.S.A.) general linear models procedure. Effects were considered significant at $P \leq 0.05$. Pairwise comparisons between LSMeans were used to detect differences between treatments. Differences were considered significant at $P \leq 0.05$. Data are presented as LSMeans ± SEM (standard error of the mean). To normalize gene expression data, fold change values were $\log_2$ transformed prior to statistical analysis. Gene data are presented as LSMeans ± SEM of non-transformed data. Some genes have standard curves, however if the gene was not identified in at least 25% of samples, the gene was recorded as not detectable.

RESULTS

Table 1 contains the p-values and sample sizes for all variables and main effects tested for genes expressed in female trout liver, white muscle, and visceral adipose tissue.

Gene Expression in Liver

Four lipogenic genes were significantly affected by ploidy; *gpat*, *acyl*, *fas*, and *scd1* had greater expression in 3N liver in November and January (Figure 1a). Triploid liver had double the expression of *acyl* and *scd1,* compared to the 2N counterparts. Triploid liver had a seven-fold

increase in *fas* expression in January. Only one of the isoforms encoding for the enzyme that controls the rate of mitochondrial β-oxidation was affected by ploidy; 2N liver had higher expression of *cpt1a* than 3N liver in November and January (Figure 1b). Two of the genes involved in peroxisomal β-oxidation of long chain fatty acids, *aco* and *acdhvl,* were increased in 3N liver compared to 2N liver, regardless of month. A two-fold higher expression level of *lpl* was observed in 3N liver in November with a three-fold higher expression level in January (Figure 1c). A two-fold increase in expression of 3N liver *me* was observed at both time points. Both 2N and 3N females had greater expression of *fabp3* in January when compared to November (Figure 1c). Triploid liver had greater expression of *redd1* and *rxr* in November, but there were no differences in ploidy in January (Figure 1d). Triploid liver had greater expression of *raptor* at both time points. November 3N liver had the greatest expression of *pparβ,* while there was greater expression of *pparγ* in 3N liver at November and January (Figure 1d). Interestingly, ration did not affect liver gene expression ($P>0.05$; data not shown).

Gene Expression in White Muscle

Diploid muscle had greater expression of *gpat* in both November and January, while 3N muscle had greater expression of *scd1* (Figure 2a). Expression of *magl* was higher in November than in January at both time points. Diploid muscle had higher expression of *cpt1a, cpt1b, cpt2, acdhvl,* and *acat2* compared to their 3N counterparts while the greatest expression of *aco* observed in January 2N (Figure 2b). Expression levels of *fabp3* and *cd36* were higher in 2N muscle at both sample periods (Figure 2c). The highest expression of *lpl* was observed in January 3N muscle (Figure 2c). Diploid muscle had greater expression of *erk* and *mo25* in November and January compared to their 3N counterparts (Figure 2d). January, 2N muscle had the highest expression of *redd1*. January also had higher expression of *pparα,* regardless of ploidy, compared to November (Figure 2d). Only two genes were affected by ration in white muscle, *erk* and *acat2* (Figure 3a; $p=0.0435$ and $p=0.0279$, respectively). The β-oxidation gene, *acat2,* and signaling protein, *erk,* had the greatest expression at the highest ration level, satiation (Figure 3a).

Gene Expression in Visceral Adipose Tissue

None of the genes involved in fatty acid synthesis were significantly affected by ploidy or month (Figure 4a). Four genes within β-oxidation had higher expression in 2N visceral adipose tissue than in the 3N counterparts for November and January (Figure 4b). The only isoform of the gene regulating the rate limiting step of mitochondrial β-oxidation to have significant month differences in visceral adipose tissue was *cpt1c;* its expression was higher in November compared to January, regardless of ploidy (Figure 4b). Triploid visceral adipose tissue had greater expression of *fabp3* compared to their 2N counterparts at both time points (Figure 4c). Diploid visceral adipose tissue had the greatest expression of *cd36* in November, while 3N fish in January had the greatest expression levels of *me* (Figure 4d).

DISCUSSION

The current study identifies genes that differ between age-matched 3N and sexually maturing 2N female rainbow trout. Time points were chosen based on previous studies using fish from the same population; we identified many significant differences between 2N and 3N females beginning at 20 M (November), and when 2N females begin to ovulate at 22 M (January) (Aussanasuwannakul et al., 2011; Aussanasuwannakul et al., 2012; Manor et al., 2012). Comparisons of ploidies used fish that were consuming identical rations, therefore differences in gene expression between 2N and 3N fish can be attributed to maturation-related signals. In contrast, differences in expression between 2N fish on different ration levels are mainly an effect of variation in nutrient supply. Although, numerous genes responded to maturation (2N vs 3N), fewer genes responded to ration level; therefore, maturation-related signals are likely a predominant factor regulating expression of genes within fatty acid metabolism and lipid repartitioning in sexually maturing fish. Furthermore, tissue-specific gene responses in maturing 2N females suggest that mechanisms regulating nutrient repartitioning are unique to each tissue. This pattern is plausible considering the specific role each tissue type has with respect to fatty acid metabolism.

Collectively, data support an increased capacity for fatty acid synthesis in 3N liver compared to maturing 2N females with increased expression of all six lipogenic genes included in this multiplex. Conversely, 2N females exhibited higher expression levels of genes in β-oxidation, including *cpt1a*, and *aco* in liver, *cpt1a, cpt1b, cpt2, aco, acat2*, *acdhvl*, and *ehhadh* muscle, and *cpt1c*, *acdhm, acdvl, and ehhadh* in visceral adipose tissue. These findings support the assertion that 2N females are oxidizing fatty acids in muscle and visceral adipose tissue to provide energy for gonadogenesis. Data further supports the assertion sterile 3N females are continuing to synthesize and store excess energy in the form of fatty acids and triglycerides because they are sterile. These findings are supported by compositional data previously published (Manor et al., 2013). Manor et al. (2013) reported decreased saturated (SFA) and monounsaturated (MUFA) fatty acid content in 2N muscle and visceral adipose tissue compared to the 3N counterparts when analyzing these same fish. Although gene expression data, along with phenotypic data, does support an up-regulation of fatty acid synthesis in 3N females and β-oxidation in 2N females during this time period, it is important to remember these pathways are primarily regulated by post-transcriptional, protein phosphorylation. Therefore, changes in expression are only suggestive of pathway regulation.

Genes for fatty acid transporters were differently expressed across all three tissues. Triploid liver had over two-fold higher expression of *lpl* and *me* while 2N muscle had greater expression of *fabp3* and *cd36* and 3N visceral adipose tissue had higher expression of *fabp3* in November and January. These data are indicative of 3N females having increased fatty acid up-take in the liver and visceral adipose tissue with the 2N females having increased transport of fatty acid within muscle. This assertion is again supported by fatty acid compositions of muscle and visceral adipose tissue with 3N females increasing and 2N females decreasing lipid stores within both of these tissues during sexual maturation (Manor et al., 2013).

Expression profiles of signaling factors were also different across tissues, but appear to correspond well with target gene expression. Triploid liver had higher expression levels of *raptor* and *pparγ.* Raptor is involved in TORC1 assembly within the mTOR signaling pathway. Both raptor and PPARγ increase transcription of lipogenic genes, which was observed in this study in 3N female liver. Conversely, 2N muscle had higher expression of three cofactors within the

mTOR signaling pathway that inhibit the assembly of TORC1, *erk, redd1,* and *mo25.* Inhibition of TORC1 prevents transcription of lipogenic genes. This assertion is supported by increased expression of β-oxidation genes within 2N muscle. Additionally, 2N visceral adipose tissue had higher levels of *erk* and *akt* expression. Both genes code for inhibitors of TORC1 assembly, suggesting there may be an inhibition of lipogenic gene transcription which is supported by increased expression of β-oxidation genes. Changes in white muscle and visceral adipose tissue fatty acid metabolism during sexual maturation do not appear to be a result of altered PPAR gene expression because these genes did not differ between 2N and 3N females. Again, it is important to remember both mTOR and PPAR signaling pathways are primarily regulated by protein phosphorylation, which was not measured in this study; therefore, gene expression is only indicative of a portion of pathway actions.

β-oxidation genes within trout liver, muscle, and visceral adipose tissue responded similarly, with 2N females exhibiting an up-regulation of this pathway in these tissues, suggesting an increase in the use of lipids as an energy source. There appears to be an inhibition of TORC1 assembly associated with an up-regulation of genes involved in the β-oxidation pathway. Two studies have used rapamycin as an mTOR inhibitor to elucidate mTOR's role in β-oxidation (Sipula et al., 2006; Brown et al., 2007). Brown et al. (2007) used cultured primary rat hepatocytes and found that inhibition of mTOR by rapamycin increased β-oxidation of exogenous fatty acids 46% at 18 hours and 100% at 48 hours. Moreover, they showed that esterification of exogenous fatty acids and *de novo* lipid synthesis was reduced by 40% and 60%, respectively (Brown et al., 2007). We reported a reduction in muscle fat content, indicative of a reduction in fatty acid synthesis and/or an increase in β-oxidation in 2N females (Manor et al., 2014). Rapamycin-inhibition of mTOR also decreased gene expression of *acc* and *gpat* (Brown et al., 2007); these genes were decreased in 2N liver when compared to 3N liver in our study as well.

Sipula et al. (2006) showed that β-oxidation is increased in L6 myotubes and in S6K1-deficient mice when mTOR is inhibited by rapamycin. These authors reported significant increases in the activities of *cpt1* and *cpt2* in culture with a subsequent increase in their mRNA levels *in vivo* (Sipula et al., 2006). We observed increases in *cpt1* and *cpt2* gene expressions;

however, we did not measure the activities of these enzymes. The mechanism by which mTOR elicits its effects on fatty acid β-oxidation remains unclear. Sipula et al. (2006) suggested that mTOR directly acts on key oxidative genes and proteins and causes a flux through the β-oxidation pathway. However, Brown et al. (2007) suggested that decreased expression of *acc* during rapamycin-inhibition of mTOR caused a decrease in its product, malonyl-CoA. Malonyl-CoA is the first intermediate in the fatty acid synthesis pathway and acts to inhibit CPT1. In the current study, we did not observe a decreased expression of *acc* in the muscle. Nevertheless, our data does support the idea that mTOR may play a role in regulating β-oxidation as well as fatty acid synthesis.

The marked up-regulation of genes involved in fatty acid synthesis in 3N females along with up-regulation of genes involved in β-oxidation in 2N females, with no effects of ration level on gene expression, suggests that sexual maturation is a dominant physiological process that alters fatty acid metabolism regardless of the level of nutrient restriction. Therefore, fatty acid metabolism is primarily regulated by maturation-related signals, such as estrogen (E2) during this time period. Estrogen elicits its effects on lipid metabolism through the estrogen receptor α (ERα) (Wend et al., 2013). Estrogen administration decreases adipocyte size and number in cultured mouse adipocytes by inducing lipolysis (Wend et al., 2013). Additionally, IGF-1 is another important regulator of sexual maturation in trout and elicits effects on lipid metabolism (Taylor et al., 2008; Sanchez-Gurmaches et al., 2012). These authors reported that there was a differential expression of *fabps* and *cd36* in response to insulin and IGF1. It is plausible maturation-associated signals, such as E2 and IGF1, could play an important role in regulating fatty acid metabolism and nutrient partitioning during sexual maturation in female trout by primarily up-regulating genes involved in β-oxidation in 2N females.

CONCLUSION

Data from this study provide information about metabolism of lipid stores during moderate feed restriction at an important life stage. Additionally, comparisons between maturing 2N and sterile 3N female rainbow trout are a unique model to study effects of maturation in fish.

In general, ration levels employed in this study did not affect expression of genes included in this multiplex. These findings suggest ration levels were not nutrient restrictive enough to alter fatty acid metabolism during this time period. Conversely, sexual maturation did have distinct effects on gene expression. Diploid females have increased expression levels of β-oxidation genes in muscle and visceral adipose tissue, while 3N females have increased expression of lipogenic and fatty acid up-take genes in liver. Increased β-oxidation in 2N females is associated with altered gene expression of mTOR cofactors that inhibit TORC1 in muscle as well as increased *pparβ* expression in visceral adipose tissue. Increased fatty acid synthesis in 3N females is associated with altered expression of mTOR cofactors that increase TORC1 and increased *pparγ* expression in the liver. Additionally, the up-regulation of genes involved in β-oxidation pathways across ration levels in all three tissues suggests maturation-induced hormonal signals, such as estrogen and IGF1, are regulators of these effects. Data suggest moderate nutrient restriction does not alter lipid repartitioning during sexual maturation or negatively impact egg quality. These findings further support the assertion that moderate nutrient restriction is an optimal feeding strategy for fish retained for additional breeding cycles, as feeding to satiation does not reduce expression levels of β-oxidation genes in 2N females during sexual maturation.

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TABLES and FIGURES

	Liver				*White Muscle*				*Visceral Adipose Tissue*			
	Ploidy	**Month**	**P x M**	**n (43)**	**Ploidy**	**Month**	**P x M**	**n (44)**	**Ploidy**	**Month**	**P x M**	**n (40)**
Fatty Acid Synthesis												
gpat	**<0.0001**	**0.0002**	0.8292	42	**0.0005**	0.411	0.8886	44	0.0645	0.549	0.3105	40
srebp1	**0.0055**	0.1609	0.1473	29	0.337	0.4315	0.7082	26	ND	ND	ND	0
acyl	**<0.0001**	**0.007**	0.3148	39	0.7753	0.8439	0.2768	44	0.3819	0.0662	0.5886	39
acc	**0.0546**	0.1296	0.3018	39	0.4286	0.8301	0.7531	36	0.1588	0.9259	0.7785	36
fas	**<0.0001**	**0.0019**	0.3833	39	0.5228	0.8742	0.6465	38	0.9942	0.1372	0.9259	40
scd1	**0.0015**	**0.0176**	0.5390	40	**0.0013**	0.235	0.7805	44	0.3571	0.0634	0.8217	35
β-Oxidation												
magl	0.5653	0.0953	0.7080	36	0.7962	**0.0203**	0.2949	43	0.8946	0.9518	0.3029	39
cpt1a	**<0.0001**	0.7687	0.5158	38	**0.0001**	0.539	0.0773	36	0.3600	**0.0311**	0.4033	30
cpt1b	ND	ND	ND	16	**0.0001**	0.2008	0.9976	44	0.8457	0.5238	0.8364	35
cpt1c	0.1357	0.5436	0.0748	43	0.7117	0.5127	0.1296	40	**0.0134**	0.4309	0.5829	39
cpt1d	ND	ND	ND	0	0.6438	0.1982	0.5183	44	0.4970	0.7752	0.9010	39
cpt2	ND	ND	ND	0	**<0.0001**	0.5331	0.6790	36	0.8378	0.3614	0.1371	11
acat2	ND	ND	ND	0	**<0.0001**	**0.0125**	0.2988	44	0.9001	0.1500	0.1270	40
acdh	ND	ND	ND	0	ND	ND	ND	0	0.1616	0.1134	0.2017	29
acdhm	ND	ND	ND	0	0.2383	0.5164	0.4608	44	**0.0035**	0.7076	0.7628	31
acdhvl	**0.0004**	0.7817	**0.0194**	39	**0.018**	0.2607	**0.0436**	36	**0.0008**	0.2775	0.2768	34
aco	**0.0004**	0.4694	0.3116	40	**0.0285**	0.1714	0.0585	43	0.0714	0.7634	0.1270	40
ehhadh	0.696	0.0895	0.6406	40	**<0.0001**	0.392	0.1560	43	**<0.0001**	0.1621	0.1082	40
Fatty Acid Transport												
fabp3	0.6455	**0.0236**	0.2725	38	**0.0034**	0.0684	0.3878	44	**0.0109**	0.2692	0.4621	39
fabp4	0.8154	0.1437	0.3046	23	ND	ND	ND	0	0.0894	0.2117	0.3029	34
lpl	**<0.0001**	0.0938	0.5148	26	**0.0033**	**<0.0001**	0.5140	41	0.2003	0.0700	0.3774	38
cd36	0.0615	0.1448	0.9396	41	**<0.0001**	0.104	0.5127	43	**0.0228**	0.5819	0.6799	35
me	**0.0062**	0.9301	0.7814	41	0.4925	0.3906	0.7077	43	**0.0518**	0.8166	**0.0383**	40
Signaling Factors												
erk	0.1055	0.1843	0.7566	26	**0.0024**	0.459	0.5987	38	**0.0086**	0.4337	0.0939	39
akt2	ND	ND	ND	0	0.1205	0.1499	0.6524	43	**0.0075**	0.4452	0.1993	39
redd1	**0.0007**	0.9077	**0.0111**	41	**0.0127**	0.0591	**0.0508**	43	0.5274	0.2651	0.8576	40
mo25	0.2679	0.3604	0.4728	43	**0.0006**	0.3702	0.5499	43	0.4222	0.5097	0.0916	40
mtor	0.8834	0.8641	0.5168	37	0.8369	0.6604	0.6603	39	0.6479	0.2682	0.4975	36
raptor	**0.0045**	0.186	0.2191	26	0.9742	0.8264	0.7939	18	ND	ND	ND	8
rictor	0.3901	0.7509	0.6161	43	0.6022	0.0643	0.8308	39	**0.0040**	0.8629	0.4913	40
pras40	0.2646	0.6899	0.5871	43	0.1957	0.6252	0.1392	44	0.6561	0.4199	0.7033	38
pparα	ND	ND	ND	0	0.7364	**0.0100**	0.8875	19	0.383	0.2246	0.6770	27
pparβ	0.428	0.1417	**0.0376**	32	0.1293	0.1224	0.9834	29	0.0123	0.6667	0.8021	40
pparγ	**<0.0001**	0.6318	0.4288	43	0.6591	0.7731	0.3553	35	0.8327	**0.0466**	0.1047	40
rxr	**0.0397**	0.1218	0.1792	36	0.3504	0.7155	0.3882	43	**0.0251**	0.9339	0.7849	40

TABLE 1: SIGNIFICANCE AND N-VALUES FOR ALL GENES TARGETED BY THE MULTIPLEX IN LIVER, WHITE MUSCLE, AND VISCERAL ADIPOSE TISSUE—RATION, PLOIDY, AND MONTH EFFECTS

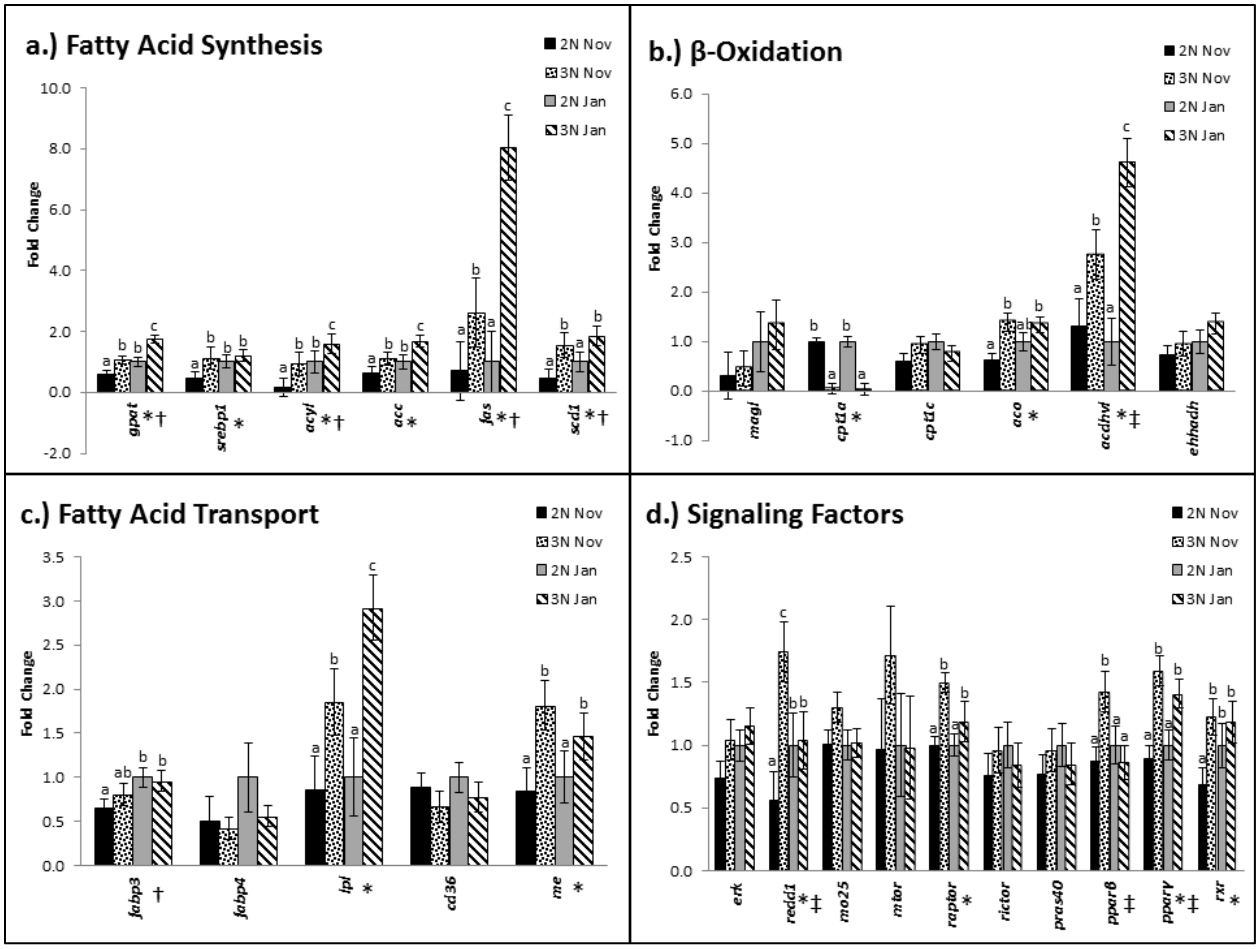

FIGURE 1: LIVER GENE EXPRESSION—PLOIDY AND MONTH EFFECTS

a.) Ploidy and month effects for liver in November and January of fatty acid synthesis genes; b.) ploidy and month effects in November and January of β-oxidation genes; c.) ploidy and month effects in November and January of fatty acid transport genes; d.) ploidy and month effects in November and January of signaling factor genes . Values are means ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Asterisks represent a significant difference between 2N and 3N fish ($P \leq 0.05$). Daggers represent a significant difference between November and January ($P \leq 0.05$). Double daggers represent a significant month-by-ploidy interaction ($P \leq 0.05$).

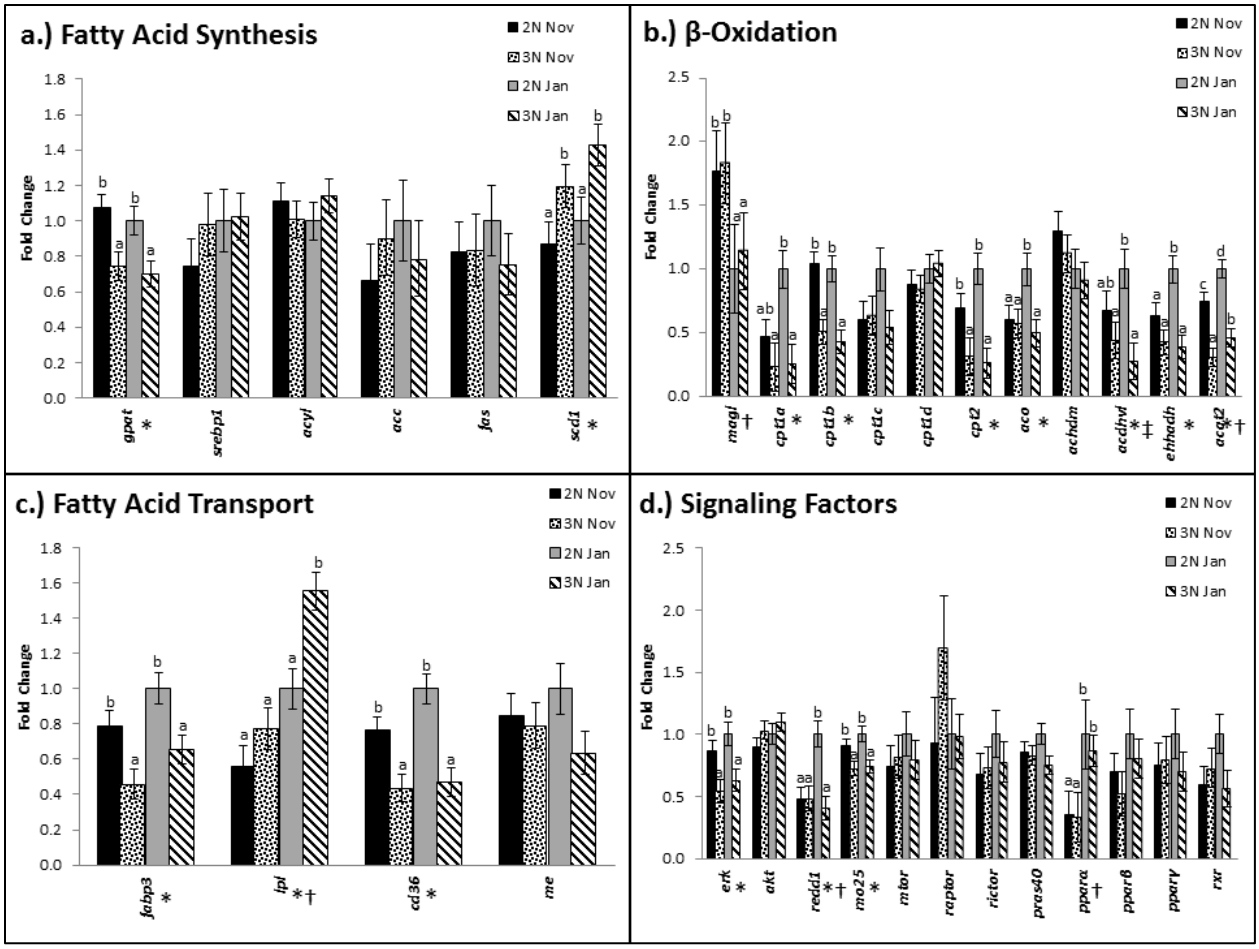

FIGURE 2: WHITE MUSCLE GENE EXPRESSION—PLOIDYAND MONTH EFFECTS

a.) Ploidy and month effects for liver in November and January of fatty acid synthesis genes; b.) ploidy and month effects in November and January of β-oxidation genes; c.) ploidy and month effects in November and January of fatty acid transport genes; d.) ploidy and month effects in November and January of signaling factor genes . Values are means ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Asterisks represent a significant difference between 2N and 3N fish (P≤0.05). Daggers represent a significant difference between November and January (P≤0.05). Double daggers represent a significant month-by-ploidy interaction (P≤0.05).

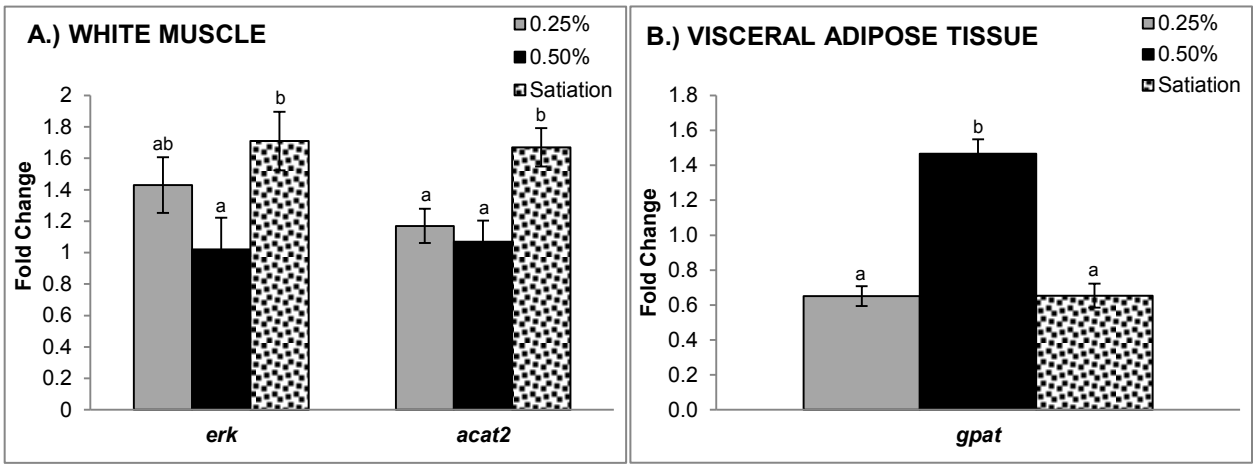

FIGURE 3: RATION EFFECTS ON GENE EXPRESSION IN MUCLE AND VISCERAL ADIPOSE TISSUE

A) Genes expressed in white muscle with significant ration effects and b) genes expressed in visceral adipose tissue with significant ration effects. Values are means ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Means without a common letter represent significant differences among mature 2N fish fed 0.25% of tank biomass/day, 0.50% of tank biomass/day, or satiation ($P \leq 0.05$).

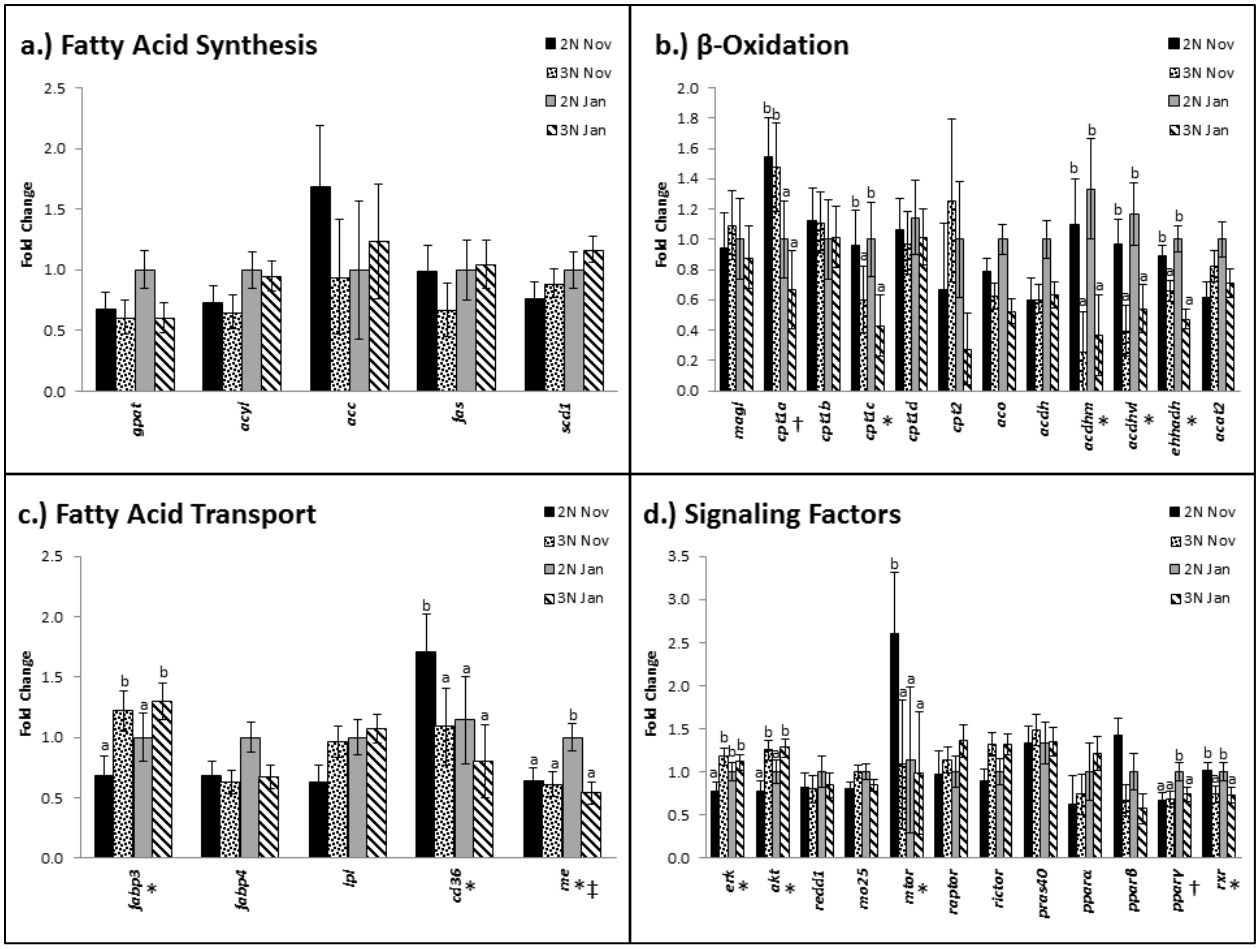

FIGURE 4: VISCERAL ADIPOSE TISSUE GENE EXPRESSION—PLOIDYAND MONTH EFFECTS

a.) Ploidy and month effects for liver in November and January of fatty acid synthesis genes; b.) ploidy and month effects in November and January of β-oxidation genes; c.) ploidy and month effects in November and January of fatty acid transport genes; d.) ploidy and month effects in November and January of signaling factor genes. Values are means ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Asterisks represent a significant difference between 2N and 3N fish (P≤0.05). Daggers represent a significant difference between November and January (P≤0.05). Double daggers represent a significant month-by-ploidy interaction (P≤0.05).

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CHAPTER 3:

Title

Expression of genes associated with fatty acid metabolism during maturation in diploid and triploid female rainbow trout

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triploid, maturation, multiplex-GeXP, fatty acid metabolism, mTOR, PPAR

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ABSTRACT

To study effects of sexual maturation on fatty acid metabolism in fish on a high nutritional plane, expression of thirty-five genes involved in fatty acid metabolism was determined in sexually maturing diploid (2N; fertile) and triploid (3N; sterile) female rainbow trout. Gene expression was assessed in liver, white muscle, and visceral adipose tissues for fish that were 16 to 24 M of age. Previously, we reported minimal differences in most growth measurements, but there were changes in muscle proximate composition, visceral fat stores, and fatty acid contents at 21 M. Here, we report that gene expression profiles of liver and white muscle corresponded to the previously measured phenotypes most closely. Differences in gene expression occurred at 20 M. Triploid females had increased expression of genes involved in fatty acid synthesis; including *gpat, srebp1, acyl, acc, fas,* and *scd1* in liver and *fas* in muscle. Conversely, 2N muscle had increased expression of β-oxidation genes *cpt1b, cpt2, ehhadh,* and *acat2* and TORC1 inhibitors *redd1, erk, mo25,* and *pras40.* Diploid muscle also had increased expression of *pparβ* along with increased expression of the fatty acid transport gene *cd36,* and β-oxidation genes *cpt1a, cpt1c, aco,* and *acdhvl* at 20 M. Additionally, 2N visceral adipose tissue had increased *cpt1a* expression at 22 M. Overall, data suggest 3N females are undergoing higher levels of fatty acid synthesis while 2N females have higher levels of β-oxidation during sexual maturation. Phenotypic data supports these findings with decreasing fatty acid stores in 2N females during this time period. Additionally, changes in gene expression are associated with altered expression within the mTOR and PPARβ signaling pathways.

INTRODUCTION

Although nutrient repartitioning is important to sexual maturation in female fish, little is understood about regulation of nutrient mobilization during this time period. Two pathways known to regulate nutrient metabolism in mammals are the mechanistic target of rapamycin (mTOR) and peroxisome proliferator activated receptors (PPAR) pathways (Laplante and Sabatini, 2011; Poulsen et al., 2012). Both pathways respond to nutrient availability and alter

target gene expression of key enzymes involved in fatty acid metabolism. Albeit, both pathways are not specific to controlling lipid metabolism; they are active in a variety of other processes such as inflammation, immune function, apoptosis, protein metabolism, and stress resistance (Laplante and Sabatini, 2011; Poulsen et al., 2012). Sexual maturation in salmonids, such as rainbow trout, is not only a period of immense restructuring of metabolism, it is also perceived as a stress (Sumpter et al., 1991). Increased energy demand during this time requires fat mobilization from muscle and visceral adipose tissue lipid stores (Nassour and Legar, 1989; Sumpter et al., 1991; Shearer, 1994; Jonsson et al., 1997; Kiessling et al., 2001; Memis and Gun, 2004; Salem et al., 2006; Gorgun and Akpinar, 2007; Riberio et al., 2011). This restructuring of metabolism to support a shift from somatic to gonadal growth and the importance of lipid metabolism during this time period makes mTOR and PPAR signaling pathways primary candidates for regulating this process.

The mTOR pathway is a central signaling cascade that plays a role in integrating energy-sensing pathways. Regulation of mTOR provides a mechanism for cells to transition between anabolic and catabolic states in response to nutrient and energy availability (Laplante and Sabatini, 2011). There are two main paths mTOR can act through; the assembly of mTOR Complex 1 (TORC1) and mTOR Complex 2 (TORC2). TORC1 elicits its effects on lipid metabolism by increasing expression of genes involved in fatty acid synthesis (Laplante and Sabatini, 2009; Caron, 2010). Whereas, TORC2 is believed to play a role in regulating the transcription of genes involved in fatty acid β-oxidation (Sipula et al., 2006; Brown et al., 2007; Jones et al., 2009). There has been some assessment of the fatty acid metabolism by investigating gene expression of *fas, srebp1,* and *cpt1* in salmonids (Lansard et al., 2009; Skiba-Cassy et al., 2009; Seiliez et al., 2011). Data suggest there are metabolic differences in nutrient utilization between fish consuming altered protein (Seiliez et al., 2011) and fishmeal-replacement diets (Lansard et al., 2009). Divergently bred lines of rainbow trout (lean and fat) also have different nutrient utilization resulting in different phenotypes (Skiba-Cassy et al., 2009). The consensus among these studies is that the mTOR signaling pathway is involved in nutrient utilization in a variety of situations ranging from genetic selection to dietary alterations. These findings further support mTOR as a primary pathway of interest when investigating regulation of fatty acid metabolism during sexual maturation in fish.

Conversely, the PPAR signaling pathway is known to respond to lipids and elicit transcriptional changes on genes involved in lipid metabolism in mammals. PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors (Poulsen et al., 2012). Gender and stage of life cycle influence expression levels of all PPARs (α, β, and γ) in brown trout (Batista-Pinto et al., 2009) with estrogen appearing to play an important role in their differential expression. PPARγ affects transcription rates of a variety of lipogenic target genes such as *fabp, cd36, lpl, leptin, acc, fas, and scd1* (Lee and Hossner, 2002). Additionally, PPARα and PPARβ are responsible for regulating fatty acid β-oxidation (Varga et al., 2011). PPARs' involvement in fatty acid metabolism makes them prime candidates as regulators of fatty acid metabolism during sexual maturation in fish.

Previous work from this same research group has investigated effects of ration level and sexual maturation on expression of thirty-five genes involved in fatty acid metabolism using Multiplex-PCR (Manor et al., 2014a). Investigating only two time points during sexual maturation provided a brief glimpse into metabolic changes that occur in lipid stores when fish are moderately feed restricted. In general, ration levels did not meaningfully affect expression of genes included in the multiplex; however, sexual maturation did have distinct effects on gene expression between 20 and 22 M (Manor et al., 2014a). It is apparent mTOR and PPAR pathways are important signaling mechanisms during sexual maturation and that maturation-related signals, such as estrogen, may be regulators of these processes. The current study investigates changes in expression of thirty-five genes involved in fatty acid metabolism in diploid (2N; fertile) and triploid (3N; sterile) fish throughout sexual maturation from 16 to 24 M. This report is part of a larger, comprehensive investigation of growth parameters, fillet quality attributes, muscle collagen, muscle protein thermal stability, and fatty acid composition of liver, muscle, visceral adipose tissue, and ovaries of the same 2N and 3N female rainbow trout (Aussanasuwannakul et al., 2011; Aussanasuwannakul et al., 2012; Manor et al., 2012; Salem et al., 2013). Understanding how genes within pathways related to fatty acid metabolism are regulated will indicate mechanisms responsible for nutrient repartitioning during sexual maturation. Furthermore, identifying critical genes and pathways associated with phenotypic

traits will enhance our knowledge of how management strategies can regulate these mechanisms for more efficient food-fish production.

MATERIALS and METHODS

Experimental Design

A two by two by six (2x2x6) factorial, randomized-complete block design was used. In this design, family (2) became the blocking variable. Independent variables included two sex conditions (fertile 2N females and sterile 3N females) and six sampling periods or harvest endpoints (16, 18, 20, 21, 22, and 24 M of age). These independent variables generated twenty-four block-by-sex condition-by harvest endpoint combinations that were randomly assigned to fish. Treatments were replicated five times with fish as replicate. Tank layout necessitated that individual fish serve as the experimental unit. For each treatment combination, five fish were randomly selected for gravimetric and morphometric measurements, and chemical analyses.

Animals

Fish care and experimentation followed guidelines outlined by the US Department of Agriculture (USDA) and the National Center for Cool and Cold Water Aquaculture (NCCCWA; USDA—Agricultural Research Service; Kearneysville, WV, U.S.A.) Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals*. Two families, each containing 2N and 3N rainbow trout, were generated and maintained at the NCCCWA. Animals were fed a commercial feed, Zeigler GOLD Floating 5.0 mm pelleted feed (42% protein, 16% fat, and 2% fiber; 316520-36-44; Zeigler Brothers, Inc.; Gardners, PA, U.S.A.), throughout the course of the experiment. Part of the daily ration was delivered by a belt feeder. At the end of the day fish were fed by hand to apparent satiation. The amount of feed delivered by the belt feeder was altered depending on appetite. From 16 to 19 M fish were fed at 1% of body weight; between 19 and 21 M, fish were fed at 0.8%; and between 21 and 24 M, fish were fed at 0.3%. Fish were initially maintained as part of stocks in five, 1.22 m diameter tanks. In July, each of the five tanks were stocked with

thirty-five fish, totaling 175 fish for this study. The thirty-five fish assigned to each tank consisted of 2N and 3N fish from each of the two families. At each sampling period, fish were shifted to a different tank to reduce biases associated with tank. Similar tank densities were maintained during the study. To avoid temperature effects, water temperatures were maintained between 12.0 and 13.5^{o}C. A simulated ambient photoperiod was maintained with artificial lighting. Passive integrated transponders (Avid Identification Systems Inc., Norco, CA, U.S.A.) were implanted in the musculature below the dorsal fin as tags for individual fish identification.

Sampling

All fish were weighed, and length (L) was measured (fork length) once a month between July (16 M post hatching) and March (24 M). In addition, five fish per treatment per block were randomly sampled at 16, 18, 20, 21, 22 and 24 M for a total of six sampling periods. This sampling rate resulted in 20 fish sampled at each time point. Fish were held off feed 24 hrs prior to sampling and were anesthetized using 150 mg/L tricaine methanesulfonate (tricane-S; Western Chemical, Inc., Ferndale, WA, U.S.A.). Liver, white muscle, and visceral adipose tissue samples were frozen in liquid nitrogen and stored at -80^{o}C until further processing. Fish were manually filleted the following day at West Virginia University's Muscle Foods Laboratory (Morgantown, WV, U.S.A.). Gravimetric and morphometric measurements and chemical analyses are reported in Aussanasuwannakul et al. (2011), Aussanasuwannakul et al. (2012), Manor et al. (2012), and Salem et al. (2013).

Gene Expression Analysis

Multiplex Analysis

The GenomeLab GeXP genetic analysis system (Beckman Coulter Inc.; Pasadena, CA, U.S.A.) was used to simultaneously analyze expression of thirty-nine genes in liver, white muscle, or visceral adipose tissue. Within the multiplex, thirty-five genes were associated with fatty acid metabolic pathways and four served as potential reference genes. Primers were designed using eXpress Designer software (Beckman Counter Inc.; Pasadena, CA, U.S.A.) and primer sequences were compared against other rainbow trout gene sequences using the BLAST function within the NCBI database to reduce unintended sequence amplification. The size of each amplicon was confirmed with its expected length. No undetermined peaks interfered with

amplification of the intended multiplex. Optimization of the multiplex, standard curve, reverse transcriptase (RT) and PCR reactions, and capillary electrophoresis were performed as recommended by the manufacturer (GeXP Chemistry protocol A29143AC; February, 2009) with reagents provided in the GeXP Start Kit (Beckman Coulter Inc.; Pasadena, CA, U.S.A.). GenBank accession numbers, database reference numbers, and references for sequences used to generate multiplex primers, the associated regulatory pathways, roles in lipid metabolism, and R^2 values for the RNA standard curve (0.2 ng /μL – 100 ng/μL), are shown in Appendix 4, Table 6. Primer sequences that include universal tags are provided in the supplementary data file as, Appendix 3, Table 5.

Areas for each peak within the multiplex were exported to eXpress Profiler software (Beckman Coulter, Inc., Pasadena, CA, U.S.A.) for analysis and normalization to the internal kanamycin control. Concentrations were interpolated from the standard curves for each gene of interest. GeNorm software was used to determine which reference genes were most stable. The most stable reference genes were *β-actin*, *rplp2*, and *ef1a* for all three tissues. M-values for these three genes and for all three tissues were below 0.5; therefore, their geometric mean was used to generate a normalization factor for each sample. Thus, the normalized expression of each gene transcript is reported as the quantity relative to the geometric mean of the selected reference genes with arbituray units.

RNA Isolation

To isolate RNA, 50 – 100 mg of tissue was homogenized in 1 mL TRIzol (Invitrogen; Carlsbad, CA, U.S.A.), per manufacturer's suggested protocol, using a 5 mm steel bead and a multi-tube shaker. The RNA pellet was washed with 75% ethanol and resuspended in nuclease-free water. RNA quality and quantity was determined by measuring absorbance at 260 nm and 280 nm.

Multiplex PCR

The multiplex reverse transcription (RT) reactions were optimized for each tissue. Reverse transcription reactions included 2 μL 5X RT buffer, 1 μL gene-specific reverse primer mix, 0.5 μL RT, and 1.25 μL kanamycin RNA (internal control) in a 10 μL reaction. Reactions

included 1.25 µL, 2.5 µL, and2.5 µL of 100 ng DNase treated RNA for liver, white muscle, and visceral adipose tissue respectively. The RT was incubated according to kit instructions. An aliquot (4.65 µL) of the resultant cDNA was used in PCR reactions for all three tissues that included 2 µL 25 mM $MgCl_2$, 2 µL 5X PCR buffer, 1 µL forward primer mix, and 0.35 µL DNA Taq polymerase. The PCR was incubated according to kit instructions. 1 µL of PCR products was combined with 38.5 µl sample loading solution and 0.5 µL size standard 400. The PCR products were separated by capillary electrophoresis in the GeXP Genetic Analysis System using a modified Frag-3 protocol with a separation voltage of 6.0 kV for 45 min.

Statistical Analysis

Data were analyzed by PROC MIXED procedure of SAS® system for Windows, version 9.1 (SAS Institute Inc., 2004). Variance components were estimated by restricted maximum likelihood method (Ramon et al., 2006) for testing fixed effects which included age, ploidy, and their interaction. The blocking variable, family, was treated as a random effect. There was no significant effect of family as block. The DDFM=KR option was used to invoke an adjustment to standard errors, test statistics, and degree of freedom approximation. The PDIFF function of LSMEANS was used to perform pair-wise comparisons. Significant differences were defined at $P \leq 0.05$. Gene data are presented as LSMeans ± SEM. Some genes have standard curves; however, if the gene was not identified in at least 25% of samples, the gene was recorded as not detectable.

RESULTS

P-values indicating main effects of age, ploidy, and age-by-ploidy interactions are reported in Table 1. Means and significant differences are reported in Figures 1-3 for liver, muscle, and adipose tissue, respectively.

Liver Gene Expression

Regardless of ploidy, expression of *raptor* and *srebp1* increased from 16-24 M, with the most rapid increase occurring from 22-24 M (figure 1a). Significant effects of age in *raptor* and *srebp1* expression were observed between 16 and 22 M, while differences in *pras40* and *ehhadh* expression were noted at 24 M (figure 1a and 1b, respectively). Expression of *cd36* showed a steady increase from 16 to 22 M (figure 1b). Main effects of ploidy were observed for genes shown in figure 1c (*erk, raptor, rictor, pras40, srebp1, cd36, aco, acdhvl,* and *ehhadh*); 3N females exhibited greater expression levels across all months when compared to their 2N counterparts. Age-by-ploidy interactions were observed for *gpat, acyl, acc, fas, scd1, pparγ, fabp3, lpl, me,* and *cpt1a* (figure 1d-1m). Expression of *gpat* was greater in 3N liver than in 2N liver only at 20 and 21 M. Furthermore, *gpat* expression increased in 2N liver during the last 3 months of sexual maturation (21-24 M). Relative expression patterns of *acyl*, *acc, fas, fabp3*, and *fas* were similar (figure 1e-1i), with 3N liver displaying an expression "spike" at 20 M. Expression in 3N liver remained higher than 2N liver throughout the remainder of the study, with the exception of the 21 M period. At 21 M expression in 3N livers often returned to 2N levels (*acyl, fabp3, scd1*). Relative expression of *ppary* (figure 1j) and *me* (figure 1l) displayed similar patterns; expression in 3N liver was greater at 20 M than in 2N liver, and remained higher for the remaining time periods. In 2N liver, *me* expression steadily decreased over time (figure 1l). Expression of *lpl* steadily increased from 16 to 24 M in 2N liver, but was significantly greater than 3N liver only at 24 M (figure 1k). Expression of *cpt1a* was significantly greater in 2N liver than in 3N liver throughout the entire sampling period (figure 1m).

Muscle Gene Expression

There was a continued decrease in *rictor* and *cpt1b* from 16 to 24 M, while *scd1* muscle expression had a "spike" in expression at 20 M (figure 2a). Diploid muscle had increased expression of *erk, mo25, pras40, fabp4, cpt1b, cpt2, ehhadh,* and *acat2* compared to 3N muscle; whereas, 3N muscle had greater expression of *fas* than 2N muscle (figure 2b). All seven genes with significant age-by-ploidy interactions have similar expression patterns (figure 2c-2i). Expression in 3N muscle was greater than (*redd1* and *acdhvl*) or similar to (*pparb*, *cd36*, *cpt1a*, *cpt1c*, *aco*) expression levels in 2N muscle at 16 M, while expression at 18 M was not different between 2N and 3N females. For all genes demonstrating an interaction, 2N muscle had a

"spike" in gene expression at 20 M, and then at 21 M, returned to and remained at levels observed in 3N muscle. However, *cd36* and *cpt1a* remained higher in 2N muscle compared to 3N muscle at time points after 21 M.

Visceral Adipose Tissue Gene Expression

Visceral adipose tissue expression of *erk* decreased between 16 and 18 M and increased between 22 and 24 M (figure 3a). Expression of *acyl* increased between 16 and 18 M then decreased between 18 and 24 M (figure 3a). There was over a 10-fold increase in *pparγ* expression between 16 and 18 M with a subsequent decrease through 24 M (figure 3a). There was an increase in *pparβ* expression between 22 and 24 M (figure 3b). Expression of *cd36* increased between 16 and 20 M followed by a decrease in expression between 20 and 21 M (figure 3b). Expression of β-oxidation genes *aco* and *ehhadh* increased from 16 to 18 M with decreasing expression through 24 M (figure 3b). Triploid expression of *erk* and *lpl* was greater than expression in 2N visceral adipose tissue (figure 3c). Three genes had significant age-by-ploidy interactions. Diploid visceral adipose tissue expression of *mo25* increased from 21 to 24 M, while 3Ns decreased between 21 and 22 M (figure 3d). Diploid *mtor* expression increased between 22 and 24 M, whereas 3N *mtor* decreased between 22 and 24 M (figure 3e). Likewise, *cpt1a* expression in 2N visceral adipose tissue increased between 22 and 24 M, while 3N visceral adipose tissue decreased from 16 to 22 M (figure 3f).

DISCUSSION

The current study identifies genes that differ between age-matched 2N and 3N female rainbow trout undergoing sexual maturation. Fish were fed to satiation daily, putting them on a high plane of nutrition and allowing differences in gene expression to be caused by variation in maturation-induced signals, such as increasing estrogen levels in 2N females compared to their 3N counterparts (Piferrer et al., 2009). Growth, composition, and fillet quality attributes from these same fish have been previously reported (Aussanasuwannakul et al., 2011; Aussanasuwannakul et al., 2012; Manor et. al., 2012; Salem et al., 2013). Additional studies have

investigated effects of ration on physiological mechanisms and fillet quality (Cleveland et al., 2012; Manor et al., 2014a; Manor et al., 2014b). Collectively, these studies indicate body lipid stores differ between 2N and 3N females beginning at 21 M of age in a manner that supports mobilization of lipid and protein from various fat depots to support gonadogenesis in 2N females. In general, gene expression profiles in liver and white muscle from this current study corresponded to previously measured phenotypes most closely, with significant differences in gene expression beginning at 20 M, one month prior to significant phenotypic differences (Aussanasuwannakul et al., 2011; Aussanasuwannakul et al., 2012; Manor et. al., 2012; Salem et al., 2013). Triploid liver had increased expression of fatty acid synthesis-related genes while 2N muscle had increased expression of β-oxidation related genes.

In the current study, increased *gpat, srebp1, acyl, acc, fas,* and *scd1* expression suggest greater fatty acid synthesis in 3N liver. These data are in agreement with previous findings (Manor et al., 2014a). Furthermore, 3N liver exhibited increased expression of *pparγ*, a transcription factor responsible for increasing expression of lipogenic genes such as *acyl, acc, fas,* and *scd1;* this finding supports our previous reports as well. Increased expression of *erk, akt*, and *raptor,* activators of TORC1 signaling, suggests mTOR signaling may also play a role in regulating fatty acid synthesis in these fish. Interestingly, *pparγ* expression is increased through TORC1 signaling (Laplante and Sabatini, 2011). On the other hand, there was also increased expression of *aco, acdhvl,* and *ehhadh,* indicating increased β-oxidation within 3N liver. These data contradict our previous findings where only fatty acid synthesis genes were up-regulated in 3N liver (Manor et al., 2014a). The up-regulation of both fatty acid synthesis and β-oxidation genes in 3N liver compared to 2N liver suggests 3N females may have increased fatty acid turnover during this time period. Nevertheless, it is important to note that fish in the current study were on a much higher plane of nutrition, with greater intramuscular and visceral fat depots when compared to our previous work (Cleveland et al., 2012; Manor et al., 2013; Manor et al., 2014a). These differences in energy reserves and rates of lipid mobilization may contribute to disparity in these two studies. Additionally, it is important to remember fatty acid metabolism is primarily regulated post-transcriptionally by protein phosphorylation; therefore, changes in expression are only suggestive of pathway regulation (Laplante and Sabatini, 2011). Perhaps increased liver, lipogenic gene expression at 20 M in 3N females may have contributed

to increased fillet yields, crude lipid, and saturated fatty acid (SFA) content of 3N fillets by 21 M.

Conversely, liver data also revealed increased β-oxidation in 2N females through increased expression of *cpt1a.* There was a shift toward decreased β-oxidation between 21 and 24 M and increased fatty acid uptake through increased *lpl* expression at 22 M. This time frame was compatible with maximum gonadosomatic indices (GSI), with most fish ovulating by 24 M (Aussanasuwannakul et al., 2011). Interestingly, there was an indication of reduced fatty acid synthesis in 2N liver through decreased *me* expression from 16 to 22 M. Increased expression of liver β-oxidation genes supports the assertion that 2N females are oxidizing fatty acids to provide energy for gonadogenesis, while increased expression of lipogenic genes suggest sterile 3N females are continuing to synthesize and store excess energy in the form of fatty acids and triglycerides (Manor et al., 2012). Although liver is not a lipid storage tissue, it is a central organ in lipid metabolism and is responsible for synthesis and β-oxidation of fatty acids. Liver is also responsible for packaging and distributing fatty acids to peripheral tissues during sexual maturation in rainbow trout (Kandemir and Polat, 2007).

In white muscle, ploidy primarily affected β-oxidation and mTOR genes. These observations suggest increased β-oxidation is associated with altered mTOR signaling and is responsible for distinct differences in 2N and 3N females. Diploid muscle gene expression suggests inhibition of TORC1 assembly is favored in muscle during sexual maturation as supported by increased expression of TORC1 inhibitors *mo25,* and *pras40*. Elevated expression of TORC1 inhibitors in 2N muscle was also observed in our previous study (Manor et al., 2014). Interestingly, *redd1,* another inhibitor of TORC1, was increased at 20 M in 2N muscle, which corresponds to the time period of rapid gonadogenesis. Subsequent increased expression of β-oxidation genes (*pparβ, cd36, cpt1a, cpt1c, aco,* and *acdhvl*) at 20 M further suggests an elevation of β-oxidation in 2N muscle. This elevation is dependent on sexual maturation because expression levels of these genes did not change over time in 3N muscle. Furthermore, these changes in gene expression in 2N muscle at 20 M correspond well with changes in growth and composition data that occurred in 2N females at 21 M; decreased crude lipid, SFA, and monounsaturated fatty acid (MUFA) contents in muscle were observed. Collectively, these data

suggest that increased β-oxidation mobilizes fatty acids from 2N muscle to support gonadogenesis.

Increased expression of *pparβ* at 20 M in 2N muscle suggests this pathway may also contribute to up-regulation of β-oxidation in 2N muscle, which is in agreement with previous findings (Batista-Pinto et al., 2009; Manor et al., 2014). Batista-Pinto et al. (2009) determined there were differences in PPAR expressions between male and female brown trout during spawning, and observed variations in *pparβ* and *pparγ* expressions in maturing female liver. Furthermore, Batista-Pinto et al. (2009) observed changes in *pparα,* a gene not detected by our multiplex-PCR. In general, data suggest that while 2N females are mobilizing muscle lipids, 3N females are storing lipids in muscle, as demonstrated by increased expression of muscle *fas* and greater muscle crude lipid stores (Manor et al., 2013). Previous data indicate the aforementioned genes are not affected by ration in maturing 2N trout (Manor et al., 2014a); therefore, maturation-inducing signals, such as estrogen, may play an important role in facilitating these changes. Additionally, increased expression of *cd36* in 2N white muscle for this study supports increased transport, as was previously suggested with increased expression of *fabp3*, *cd36*, and *lpl* (Manor et al., 2014a). Altered expression of these genes involved in fatty acid transport and uptake could be responsible for decreased SFA and MUFA contents of 2N muscle in both studies (Manor et al., 2014 and 2013).

Visceral adipose tissue exhibited a slightly different gene expression profile with fewer ploidy and age-by-ploidy interactions. There is evidence that increased β-oxidation in 2N females may be responsible for decreased visceral fat content in maturing 2N females. Increased β-oxidation is suggested by increased *cpt1a* expression; this enzyme catalyzes the rate limiting step in β-oxidation. Triploid females did have an increased expression of *lpl,* supporting the assertion that 3N females are taking up excess dietary fatty acids into their visceral adipose tissue for storage causing large visceral fat deposits in these fish (Manor et al., 2012).

CONCLUSION

Sexual maturation is a dominant physiological process that causes a shift from somatic growth to gonadal growth. This study provides information about fatty acid metabolism in female rainbow trout during an important life stage. In general, genes related to fatty acid metabolism were most often differentially expressed in 2N and 3N liver and muscle beginning at 20 M, which is when effects of sexual maturation on fillet quality and nutrient repartitioning became evident. Triploid liver had increased expression of fatty acid synthesis-related genes, while 2N muscle had increased expression of β-oxidation related genes. In addition, it appears PPAR signaling is involved in changes in fatty acid metabolism during sexual maturation, especially in liver and muscle. Additionally, gene expression within the mTOR signaling pathway is altered in maturing 2N fish and may also contribute to metabolic differences between 2N and 3N female trout. Understanding the regulation of fatty acid metabolism in 2N and 3N fish is pertinent so the industry can use 2N and 3N females to their full production potential by maximizing energy use and profitability such as in determining optimal diet formulations, feeding strategies, and harvest endpoints when producing larger trout.

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TABLES and FIGURES

	Liver (n=71)				White Muscle (n=70)				Visceral Adipose Tissue (n=60)			
Gene	**n**	**Age**	**Ploidy**	**A*P**	**n**	**Age**	**Ploidy**	**A*P**	**n**	**Age**	**Ploidy**	**A*P**
Fatty Acid Synthesis												
gpat	71	**0.0104**	**0.0006**	**0.0168**	68	0.6184	0.0590	0.4326	47	0.3290	0.7035	0.4447
srebp1	61	**0.0014**	**0.0067**	0.2057	NA	NA	NA	NA	NA	NA	NA	NA
acyl	70	**<0.0001**	**<0.0001**	**0.0010**	69	0.1953	0.1148	0.5672	43	**0.0480**	0.1637	0.7512
acc	70	**<0.0001**	**<0.0001**	**<0.0001**	67	0.1773	0.4353	0.0677	55	0.5985	0.7273	0.6430
fas	61	**0.0009**	**<0.0001**	**0.0197**	67	0.1633	**0.0070**	0.6326	59	0.1624	0.1768	0.4424
scd1	67	**<0.0001**	**0.0036**	**0.0029**	60	0.0512	0.7390	0.9137	NA	NA	NA	NA
β-Oxidation												
magl	70	0.7197	0.3661	0.1666	70	0.9659	0.7336	0.6719	55	0.2289	0.8480	0.3639
cpt1a	67	**0.0005**	**<0.0001**	**0.0367**	70	0.2512	**<0.0001**	**0.0145**	38	**0.0353**	**0.0064**	**0.0087**
cpt1b	NA	NA	NA	NA	69	**0.0037**	**0.0004**	0.6108	NA	NA	NA	NA
cpt1c	71	0.2022	0.0697	0.6202	70	0.0559	0.1771	**0.0371**	58	0.7920	0.7172	0.1698
cpt1d	NA	NA	NA	NA	66	0.4380	0.8544	0.8977	55	0.8351	0.8209	0.3344
cpt2	53	0.3080	0.2071	0.5283	69	0.4782	**<0.0001**	0.1050	NA	NA	NA	NA
aco	71	0.1179	**<0.0001**	0.2078	70	**0.0075**	**0.0015**	**0.0217**	60	**0.0234**	0.8927	0.8432
acdhvl	68	0.5055	**<0.0001**	0.4752	69	**0.0199**	**0.0019**	**<0.0001**	56	0.5353	0.6241	0.0992
ehhadh	71	**0.0225**	**0.0055**	0.0619	70	0.2626	**0.0238**	0.1475	60	**0.0320**	0.4549	0.8619
acat2	53	0.4798	0.3946	0.6052	69	0.8517	**0.0087**	0.7597	51	0.1438	0.1424	0.1927
Fatty Acid Transport												
fabp3	70	**0.0014**	**<0.0001**	**0.0083**	39	0.9195	0.1965	0.7752	32	0.5361	0.3197	0.1541
fabp4	49	0.0912	**0.0204**	0.1135	48	0.5927	**0.0005**	0.6548	44	0.4623	0.1564	0.1665
lpl	66	**0.0128**	0.0635	**0.0460**	44	0.6832	0.8674	0.0612	44	0.1327	**0.0455**	0.1776
cd36	71	**<0.0001**	**0.0336**	0.0979	69	0.1717	**<0.0001**	**0.0002**	52	**<0.0001**	0.2464	0.3614
me	71	0.0970	**<0.0001**	**0.0123**	70	0.2472	0.2562	0.1283	47	0.1298	0.5006	0.9487
Signaling Factors												
erk	71	0.6891	**0.0354**	0.0816	69	0.3019	**<0.0001**	0.4809	53	**<0.0001**	**0.0290**	0.7603
redd1	71	0.1037	0.7023	0.1092	70	**0.0109**	0.2821	**0.0003**	60	0.8703	0.6145	0.1207
mo25	71	0.1067	0.1817	0.7233	70	0.1996	**0.0287**	0.3004	60	0.1901	0.5075	**0.0494**
mtor	70	0.1096	0.1820	0.8023	69	0.2559	0.5038	0.3818	59	0.7747	0.4265	**0.0503**
raptor	69	**<0.0001**	**0.0141**	0.5946	NA	NA	NA	NA	30	0.1230	0.7006	0.4036
rictor	71	0.2394	**<0.0001**	0.2167	70	**0.0361**	0.0991	0.1003	52	0.1086	0.5713	0.2075
pras40	41	**0.0111**	**0.0225**	0.4226	70	0.1548	**0.0248**	0.2135	54	0.9577	0.3866	0.7850
pparβ	61	0.4736	0.5314	0.2265	68	**0.0265**	**0.0173**	**0.0153**	60	**0.0403**	0.1607	0.2176
pparγ	71	**0.0003**	**<0.0001**	**0.0364**	NA	NA	NA	NA	33	**0.0016**	0.7962	0.7520
rxr	69	0.7637	0.3196	0.1833	70	0.0584	0.1196	0.0586	58	0.1265	0.5622	0.1738

TABLE 1: SIGNIFICANCE AND SAMPLE SIZES FOR ALL GENES TARGETED BY THE MULTIPLEX IN LIVER, WHITE MUSCLE, AND VISCERAL ADIPOSE TISSUE OF 2N AND 3N FEMALE TROUT

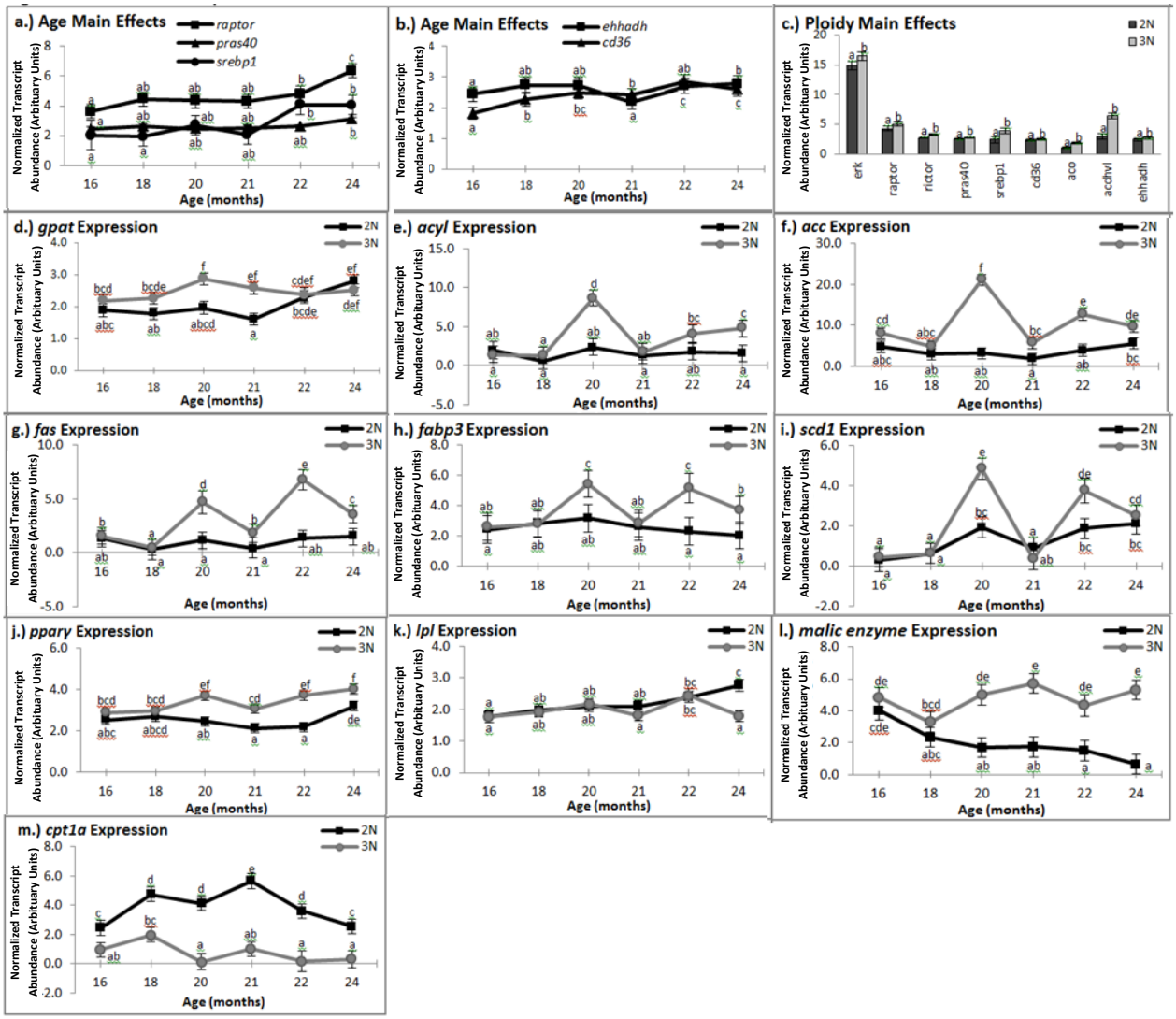

FIGURE 1: LIVER GENE EXPRESISON IN MATURING 2N AND 3N FEMALE TROUT

A and B) Age effects in liver tissue. Letters indicate significant differences between endpoints within each gene. C) Ploidy effects in liver tissue. Letters indicate significant differences between ploidies within each gene. D-M) Age-by-Ploidy interactions in liver tissue. Only genes with significant effects are shown ($P \leq 0.05$). Values are means ± SEM and represent the normalized transcript abundance, relative to the normalized mean of three reference genes (*β-actin, ef1a, and rplp2)*.

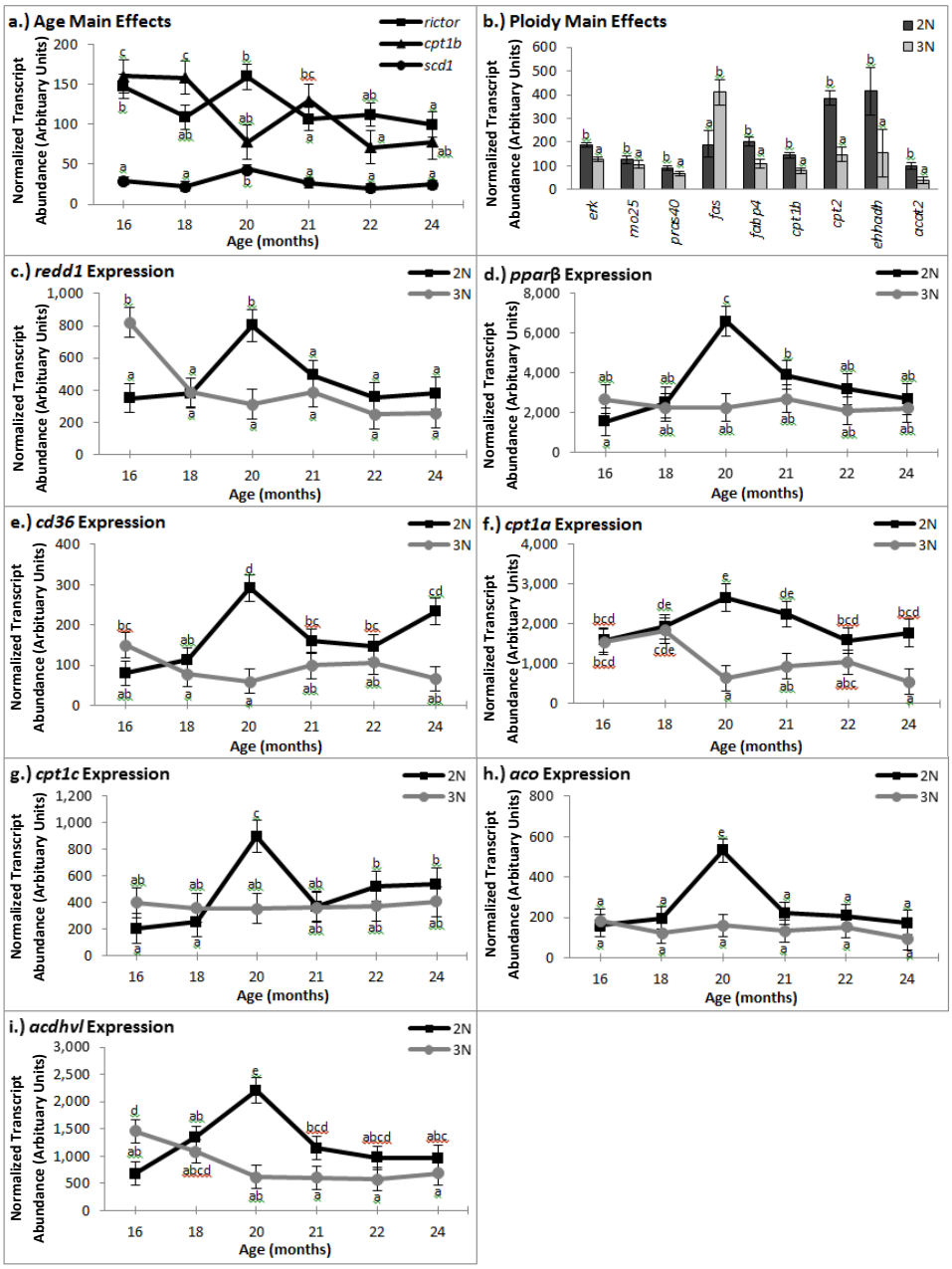

FIGURE 2: MUSCLE GENE EXPRESISON IN MATURING 2N AND 3N FEMALE TROUT

A) Age effects in muscle tissue. Letters indicate significant differences between endpoints within each gene. B) Ploidy effects in muscle tissue. Letters indicate significant differences between ploidies within each gene. C-L) Age-by-Ploidy interactions in muscle tissue. Only genes with significant effects are shown ($P \leq 0.05$). Values are means ± SEM and represent the normalized transcript abundance, relative to the normalized mean of three reference genes (*β-actin, ef1a, and rplp2)*.

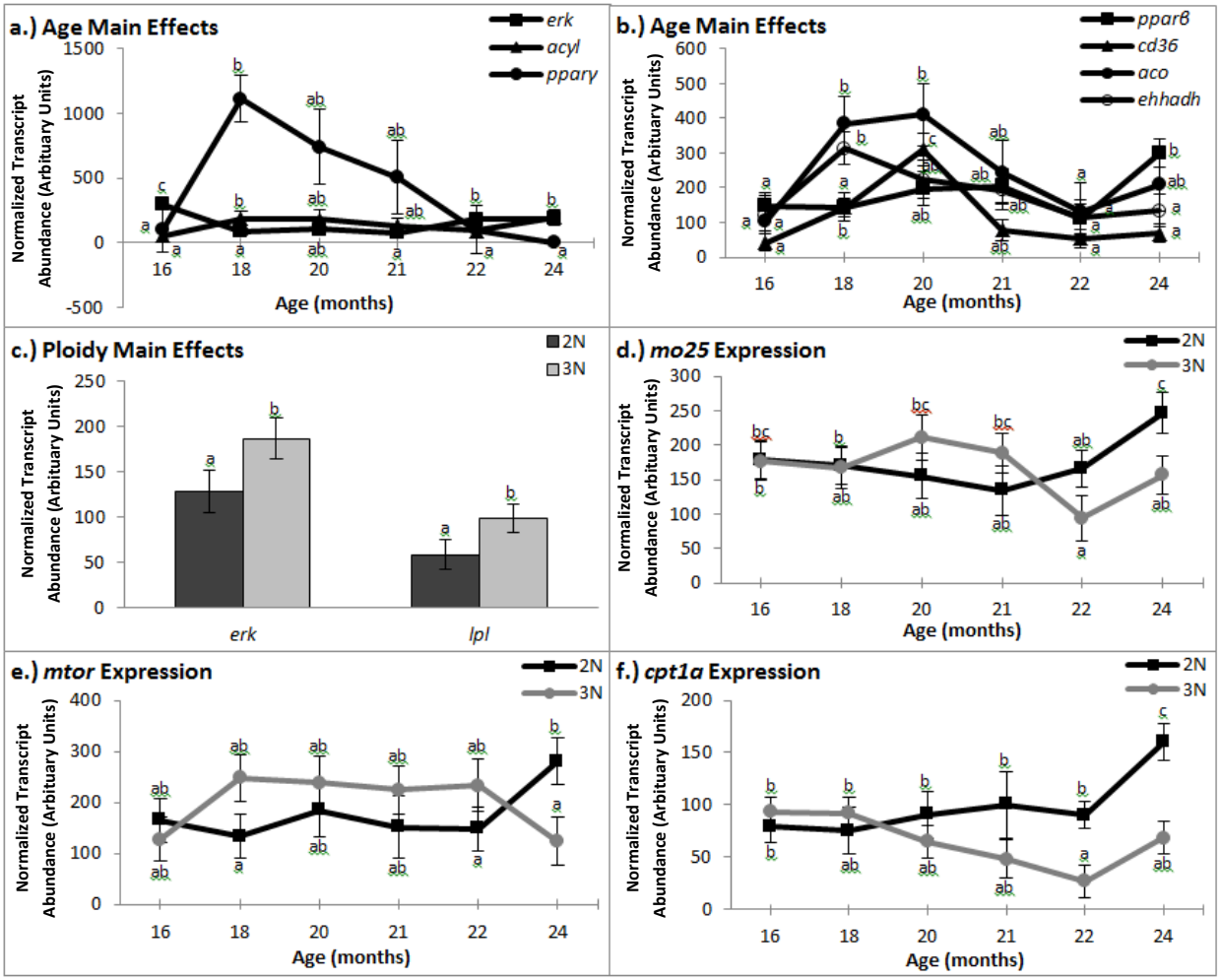

FIGURE 3: VISCERAL ADIPOSE TISSUE GENE EXPRESSION IN MATURING 2N AND 3N FEMALE TROUT

A) Age effects in visceral adipose tissue. Letters indicate significant differences between endpoints within each gene. B) Ploidy effects in visceral adipose tissue. Letters indicate significant differences between ploidies within each gene. C-L) Age-by-Ploidy interactions in visceral adipose tissue. Only genes with significant effects are shown ($P \leq 0.05$). Values are means ± SEM and represent the normalized transcript abundance, relative to the normalized mean of three reference genes (*β-actin, ef1a, and rplp2)*.

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CHAPTER 4:

Title

Differences in growth, fillet quality, and fatty acid metabolism-related gene expression between juvenile male and female rainbow trout

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ABSTRACT

Sexual maturation occurs at the expense of stored energy and nutrients, including lipids; however, little is known regarding gender effects on nutrient regulatory mechanisms in rainbow trout prior to maturity. Thirty-two, 14 month old, male and female rainbow trout were sampled for growth, carcass yield, fillet composition, and gene expression of liver, white muscle, and visceral adipose tissue. Growth parameters, including gonadosomatic index, were not affected by gender. Females had higher percent separable muscle yield, but there were no gender effects on fillet proximate composition. Fillet shear force indicated females produce firmer fillets than males. Male livers had greater expression of three cofactors within the mTOR signaling pathway that act to inhibit TORC1 assembly; *mo25,* r*ictor*, and *pras40.* Male liver also exhibited increased expression of β-oxidation genes *cpt1b* and *ehhadh.* These findings are indicative of increased mitochondrial β-oxidation in male liver. Compared to males, females exhibited increased expression of the mTOR cofactor *raptor* in white muscle and had higher expression levels of several genes within the fatty acid synthesis pathway; including *gpat, srebp1, scd1,* and *cd36*. Female muscle also had increased expression of β-oxidation genes *cpt1d* and *cpt2*. Increased expression of both fatty acid synthesis and β-oxidation genes suggests female muscle may have greater fatty acid turnover. Differences between genders were primarily associated with variation of gene expression within the mTOR signaling pathway. Overall, data suggests there is differential regulation of gene expression in male and female rainbow trout tissues prior to onset of sexual maturity that may lead to nutrient repartitioning during maturation.

INTRODUCTION

Generally, rainbow trout are sexually differentiated by 18 days post-hatch for females and 28 days post-hatch for males (Billard, 1992). This process is controlled by sex genes and it is their actions that mediate the biochemical sex inducers, such as estrogen and testosterone, to induce gonadal differentiation (Yamamoto, 1969). Studies have shown that sex-biased differences in gene expression are present after sex determination and differentiation has taken

place in trout (Hale et al., 2011). Sex bias in gene expression has been documented in multiple species including fruit flies, mice, and zebrafish (McIntyre et al., 2006; Yang et al., 2006; Santos et al., 2007). These transcriptomic studies have found there are more genes up-regulated in males than in mature female zebrafish and drosophila (McIntyre et al., 2006; Yang et al., 2006; Santos et al., 2007). Patterns of sex-biased gene expression are variable between and within species and are dependent on tissue type and developmental stage examined (Hale et al., 2011). Most of the data available for fish is from gonadal tissue of sexually mature adults (Wen et al., 2005; Santos et al., 2007). There is some data on gender differences in gene expression of brown trout liver (Batista-Pinto et al., 2009) and zebrafish muscle (Wen et al., 2005). In general, there is little data on differences in gene expression of immature fish limiting our knowledge of regulatory mechanisms that may control sexual maturation (Hale et al., 2011). Additionally, few studies have included genes involved in fatty acid metabolism (Batista-Pinto et al., 2009; Hale et al., 2011). Typically, trout are harvested prior to the onset of sexual maturation to prevent negative impacts on lipid composition and fillet quality. Therefore, an examination of gene expression and muscle composition would be beneficial in understanding lipid metabolism at an early life stage, which is relevant to fillet quality. Moreover, this type of data will assist in describing genders differences in immature fish at the molecular level and identify potential pathways that play an important role in determining gender differences in growth, fillet quality, and fatty acid metabolism.

In salmonid production fillet lipid content is an important attribute affecting the nutritional value, mechanical texture, and sensory characteristics of the fillet (Quillet et al., 2005). There appears to be an association between energy storage, increased body lipid content, and early maturity (Silverstein et al., 1997; Shearer and Swanson, 2000; Quillet et al., 2005). There is also dramatic nutrient repartitioning away from fatty acid synthesis for energy storage towards β-oxidation to supply energy for gonadogeneis during maturation (Manor et al., 2013). However, little is known regarding gender effects on nutrient regulatory mechanisms in rainbow trout prior to maturity. There are two signaling pathways that are known to be involved in growth and development as well as in fatty acid metabolism; mechanistic target of rapamycin (mTOR) and peroxisome proliferator activated receptors (PPAR) signaling pathways. The mTOR pathway is a central signaling cascade that plays a role in integrating energy-sensing

pathways (Laplante and Sabatini, 2011). The mTOR signaling pathway in fish is less characterized than in mammals; nevertheless, the consensus has been that the mTOR signaling pathway is highly conserved among species through limited *in vitro* and *in vivo* studies (Plagnes-Juan et al., 2008; Seiliez et al., 2008; Lansard et al., 2009; Lansard et al., 2010; Seiliez et al., 2011). There are limited studies in salmonids examining the role of mTOR in fatty acid metabolism, gene expression (Skiba-Cassy et al., 2009). Conversely, the PPAR signaling pathway responds to lipids and elicits transcriptional changes on genes involved in fatty acid metabolism in mammals, but gene expression studies are limited in salmonids. Gender and stage of life cycle influence expression levels of all PPARs in brown trout liver; estrogen appears to play an important role in the differential expression of PPARs (Batista-Pinto et al., 2009). Activation of PPARγ specifically leads to increased triglyceride accumulation in muscle and liver (Pouslen et al., 2012). PPARγ affects transcription rates of a variety of lipogenic target genes such as *fabp, cd36, lpl, leptin, acc, fas, and scd1* (Lee and Hossner, 2002). Conversely, PPARα and PPARβ are responsible for regulating fatty acid β-oxidation (Varga et al., 2011).

Our previous work has investigated differences in gene expression between diploid and triploid female trout and effects of ration level in maturing female trout (Manor et al., 2014a; Manor et al., 2014b). The objective of this study was to determine differences in growth, fillet quality, and the associated gene expression in immature male and female rainbow trout. Aspects of fillet quality investigated in this study include proximate composition, instrumental texture, color, and fatty acid content. In addition, relative expression levels of thirty-five multiplexed genes within the fatty acid synthesis and β-oxidation pathways were analyzed to determine if the physiological basis for differences in male and female growth and fillet quality are associated with differential regulation of genes within the mTOR and PPAR pathways. Gene expression profiles of liver, white muscle, and visceral adipose tissue were assessed. It is hypothesized that data from this study will show discrete differences in fillet quality and gene expression between immature male and female trout and identify possible gene-regulatory pathways involved in developing these differences.

MATERIALS and METHODS

Animal Care

Fish care and experimentation followed the guidelines outlined by the US Department of Agriculture (USDA) and National Center for Cool and Cold Water Aquaculture (NCCCWA; U.S. Department of Agriculture, Agriculture Research Service, Kearneysville, WV, U.S.A.) Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals.* Fish were hatched in March and reared at the NCCCWA. Passive integrated transponders (Avid Identification Systems Inc., Norco, CA, U.S.A.) were inserted in the left dorsal musculature at the fingerling stage. Fish were maintained indoors, under simulated ambient photoperiod, and supplied with partially recirculated treated spring and well water throughout the study. Water temperatures ranged from 12.4 to 14.0^{o}C. Fish were fed Zeigler G, floating, 5.0mm (3/16") pelleted feed (42% protein, 16% fat, 2% fiber; Zeigler Brothers, Inc.; Gardners, PA, U.S.A.) dispensed by automatic feeders (Arvotec, Huutokoski, Finland) that adjust feeding daily based on the predicted mass of the fish in the tank. Feeders dispensed feed in multiple feeding events between 7 am and 2 pm. Fish from each tank were weighed monthly to maintain the accuracy of the feeder system.

Sample Collection

Fish were held off feed for five days prior to sampling and were harvested using an overdose of tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, CA, U.S.A.) at 300 mg/L. Body weights were recorded. Gonads, liver, and gastrointestinal tract (GtSI; alimentary canal with associated visceral adipose tissue) were removed and weighed. Subsamples of dorsal muscle, liver, and visceral adipose tissue were immediately frozen in liquid nitrogen and stored at -80^{o}C for gene expression. Frozen liver and visceral adipose tissue samples were also used for compositional analysis. Fish were processed the following day at West Virginia University Meats Laboratory (Morgantown, WV, U.S.A.). Boneless, skinless fillets were removed from each fish and weighed. Fillet quality measurements were taken from the right fillet and the left filet was frozen at -20^{o}C until powdered in liquid nitrogen for compositional analysis.

Growth Parameters

Whole body weight (WBW) was used to calculate gonadosomatic index (GSI; gonad weight as a percent of WBW), hepatosomatic index (HSI; liver weight as a percent of WBW), and gastrosomatic index (GtSI; digestive tract and the associated visceral adipose tissue as a percent of WBW). Thermal growth coefficient (TGC) was calculated for each fish as $[(\sqrt[3]{W_f} - \sqrt[3]{W_i})/(T \times t)] \times 1{,}000$, where W_f and W_i are 13-month WBW and 10-month WBW, respectively, T is water temperature (°C), and *t* is time in days (Iwama & Tautz, 1981; Jobling, 2003). A constant 13°C was assumed for all TGC calculations because all tanks were supplied with water from the same source and water temperature did not vary substantially during this growth period.

Feed intake was measured in triplicate on individual fish over a two-week period at 12.8 months of age (mean body weight = 860 grams) as a means to estimate feed efficiency. Un-pelleted meal from the commercial diet was sampled from the manufacturer, labeled with approximately 0.2% w/w of 0.4-0.6 mm diameter leaded-glass ballotini beads (Sigmund Linder GmbH, Warmensteinach, Germany), and pelleted and oil coated at the Bozeman Fish Technology Center (Bozeman, MT, U.S.A.) in a manner similar to that used to manufacture the unlabeled commercial diet. On each of three days occurring one week apart, fish were fed the labeled feed using the same automated feeders and at the same feeding rate compared to the normal diet. The labeled feed was fed for the first half of the day (i.e., 3.5 hours), after which feeding stopped and the fish were weighed and imaged using x-ray. The x-ray opaque ballotini beads were counted from each radiograph and used to estimate feed intake. Feed efficiency was estimated as gain in body weight ÷ weight of feed consumed, whereby gain in body weight represents total body weight gain during the two-week period and weight of feed consumed was calculated as the average of three repeated intake measurements and extrapolated over the two-week period.

Fillet Characteristics

Fillet Yields: Separable muscle is reported as a percent of WBW. Trim included the head, axial skeleton, belly flap, and skin and is reported as a percent of WBW. Fillet thickness was measured at the thickest portion of the right fillet with digital calipers. Belly-flap thickness

for each fish was measured using digital calipers at three standardized locations along the length of the ventral midline; just cranial to the pectoral fin, just caudal to the pelvic fin, and at the vent. Thickness was reported as the average of the three measurements.

Fillet quality: Fresh fillet surface color was recorded with a chromameter (Minolta, Model CR-300; Minolta Camera Co., Osaka, Japan). This instrument was color calibrated using a standard white plate No. 21333180 (CIE Y 93.1; x 0.3161; y 0.3326), and L* (lightness), a* (redness), and b* (yellowness) values were recorded on the cranial and caudal ends of the right fillet.

Instrumental Texture: Texture analysis was done on 8x4cm fillet sections that were taken from musculature centered on the lateral line, 2–3 cm caudal to the pectoral girdle on the right fillet. Sections were thermally processed in a microprocessor-controlled smoke oven (Model CVU-490; Enviro-Pak, Clackamas, OR, U.S.A.) at 82^{o}C, and the cooking process was stopped when the internal temperature reached 65.5^{o}C. This cooking temperature was selected according to the USDA recommended, minimum internal temperature for fish to achieve a safe temperature without overcooking (Nilsson and Ekstrand, 1995). Total cooking time was approximately 45 minutes. After cooking, the product was allowed to cool to room temperature. Cook loss was calculated as [100-(cook weight/raw weight)x100]. Instrumental texture was measured using a 5-blade, Allo-Kramer shear attachment mounted to the TA-HDi® Texture Analyzer (Texture Technologies Corporation; Scarsdale, NY, U.S.A.), which was equipped with a 50kg load cell and ran at a crosshead speed of 127 mm/min. Shear force was applied perpendicular to the muscle fiber orientation. Force deformation graphs were recorded; average peak force (peak force / gram of sample), and total energy of shear (grams/mm) was determined using the Texture Expert Exceed software (version 2.60; Stable Micro Systems Ltd., Surrey, UK).

Proximate Analysis: The left fillet was frozen in liquid nitrogen, powdered using a Waring commercial grade blender (Model 51BL31; Waring Commercial; Torrington, CT, U.S.A.), and stored at -80°C until analysis. Liver and visceral adipose tissue samples were minced at the time of analysis. Moisture, crude lipid, crude protein, and ash analyses were completed using AOAC approved methods (AOAC, 2000). Moisture content was determined by

weighing the sample before and after an 18 hr drying period at 110 °C. Crude lipid content was determined using Soxhlet extraction with petroleum ether. Crude protein was calculated by converting percent Kjeldahl nitrogen to crude protein using 6.25 as the conversion factor. (Kjeltec^{TM} 2300; Foss North America; Eden Prairie, MN, U.S.A.). Ash was determined by incinerating the samples at 550^{o}C in a type A1500 furnace (F-A1525M-1; Thermolyne Corporation; Dubuque, IA, U.S.A.).

pH: Five grams of powdered raw muscle were mixed with 25-mL distilled water, and pH was measured using a flat surface combination electrode (pH/ion analyzer 350; Corning Inc., NY, U.S.A.). Duplicate measurements were averaged and used as the observation for that sample.

Fatty Acid Analysis: Total lipids were extracted from muscle tissue according to Bligh and Dyer (1959) using a chloroform-methanol mixture (2:1 v/v). A 0.5 gram sample of powdered muscle was used for fatty acid analysis. Fatty acids were methylated using the method described by Fritshe and Johnston (1990). Nonadecanoic acid (19:0) was used as an internal standard. Fatty acid methyl esters (FAMEs) were quantified using a Varian CP-3800 Gas Chromatograph (Varian Analytical Instruments; Walnut Creek, CA, U.S.A.) equipped with a flame ionization detector. A wall-coated, open-tubular (WCOT) fused silica capillary column (100-m length, 0.25-mm inside diameter; Varian Inc., Walnut Creek, CA, U.S.A.) was used to separate FAMEs. The stationary phase was CP-Sil 88, and nitrogen was the carrier gas at a flow of 0.3mL/min. A 10 to 1 split ratio was applied for all samples. An oven temperature of 140^{o}C for 5 minutes, followed by a temperature ramp of 3^{o}C/min to 235^{o}C, was used; 235^{o}C was held for 15 minutes. The total separation time per sample was 68.5 minutes. Injector (11-77 injector, Varian Inc., Walnut Creek, CA, U.S.A.) and detector (Flame Ionization Detector-FID, Varian Inc., Walnut Creek, CA, U.S.A.) temperatures were maintained at 270^{o}C and 300^{o}C, respectively. FAMEs were identified based on comparison to retention times of standard FAMEs (SupelcoTM quantitative standard FAME 37; Sigma-Aldrich, St. Louis, MO, U.S.A.). Peak area counts were computed by an integrator using the Star GC workstation version 6 software (Varian Inc., Walnut Creek, CA, U.S.A.).

Gene Expression Analysis

Multiplex Analysis: The GenomeLab GeXP genetic analysis system (Beckman Coulter Inc.; Pasadena, CA, U.S.A.) was used to simultaneously analyze expression of thirty-nine genes in liver, white muscle, or visceral adipose tissue. Within the multiplex, thirty-five genes were associated with fatty acid metabolic pathways and four served as potential reference genes. Primers were designed using eXpress Designer software (Beckman Counter Inc.; Pasadena, CA, U.S.A.), and primer sequences were compared against other rainbow trout gene sequences using the BLAST function within the NCBI database to reduce unintended sequence amplification. The size of each amplicon was confirmed with its expected length. No undetermined peaks interfered with amplification of the intended multiplex. Optimization of the multiplex, standard curve, reverse transcriptase (RT) and PCR reactions, and capillary electrophoresis were performed as recommended by the manufacturer (GeXP Chemistry protocol A29143AC; February, 2009) with reagents provided in the GeXP Start Kit (Beckman Coulter Inc.; Pasadena, CA, U.S.A.). Primer sequences that include universal tags are provided Appendix 3, Table 5. GenBank accession numbers, database reference numbers, and references for sequences used to generate multiplex primers, the associated regulatory pathways, roles in lipid metabolism, and R^2 values for the RNA standard curve (0.2 ng /µL – 100 ng/µL), are shown in Appendix 4, Table 6.

Areas for each peak within the multiplex were exported to eXpress Profiler software (Beckman Coulter, Inc.; Pasadena, CA, U.S.A.) for analysis and normalization to the internal kanamycin control. Concentrations were interpolated from the standard curves for each gene of interest. Data were normalized to the highest expressing sample for input into GeNorm software to determine which reference genes were most stable. The most stable reference genes were *βactin*, *rplp2*, and *ef1a* for all three tissues. M-values for these three genes and for all three tissues were below 0.5; therefore, their geometric mean was used to generate a normalization factor for each sample. Thus, the normalized expression of each gene transcript is reported as the quantity relative to the geometric mean of the selected reference genes.

RNA Isolation: To isolate RNA, 50 – 100 mg of tissue was homogenized in 1 mL TRIzol (Invitrogen; Carlsbad, CA, U.S.A.), per manufacturer's suggested protocol, using a 5 mm steel bead and a multi-tube shaker. The RNA pellet was washed with 75% ethanol and resuspended in

nuclease-free water. RNA quality and quantity was determined by measuring absorbance at 260 nm and 280 nm.

Multiplex PCR: The multiplex reverse transcription (RT) reactions were optimized for each tissue. Reverse transcription reactions included 2 µL 5X RT buffer, 1 µL gene-specific reverse primer mix, 0.5 µL RT, and 1.25 µL kanamycin RNA (internal control) in a 10 µL reaction. Reactions included 1.25 µL, 2.5 µL, and 2.5 µL of 100 ng DNase treated RNA for liver, white muscle, and visceral adipose tissue respectively. The RT was incubated according to kit instructions. An aliquot (4.65 µL) of the resultant cDNA was used in PCR reactions for all three tissues that included 2 µL 25 mM $MgCl_2$, 2 µL 5X PCR buffer, 1 µL forward primer mix, and 0.35 µL DNA Taq polymerase. The PCR was incubated according to kit instructions. 1 µL of PCR products was combined with 38.5 µl sample loading solution and 0.5 µL size standard 400. The PCR products were separated by capillary electrophoresis in the GeXP Genetic Analysis System using a modified Frag-3 protocol with a separation voltage of 6.0 kV for 45 min.

Statistical Analysis: There were 16 male and 16 female fish included in this study (2 fish x 2 genders x 8 families). All data were analyzed using analysis of variance to test for main effects of gender with PC-SAS (Version 9.1) general linear models procedure. Effects were considered significant at $P \leq 0.05$. Data are presented as LSMeans ± SEM (standard error of the mean). To normalize gene expression data, fold change values were $\log_2$ transformed prior to statistical analysis. Gene data are presented as LSMeans ± SEM (standard error of the mean) of non-transformed data. All genes have standard curves; however, if the gene was not identified in at least 25% of the samples, the gene was recorded as not detectable.

RESULTS

Growth Parameters

There were no significant differences between males and females in any of the growth parameters measured in this study including WBW, GSI, HSI, GtSI, TGC and feed efficiency (Table 1; P>0.05). However, there was a trend for females to be larger than males; 1022±48g and 993±65g, respectively (P=0.0656). The GSI values indicate fish were immature with small gonads suggesting any subsequent differences in phenotypic characteristics likely result from inherent effects of gender and not onset of sexual maturation.

Fillet Characteristics

Yields and Quality: Females did yield a higher percent separable muscle and thicker fillets than males; however, there were no significant differences between the sexes in any of the other yield measurements including trim loss, belly flap thickness, or cook loss (Table 2). There were no significant differences in fillet moisture, crude fat, crude protein, or ash content (P>0.05). Additionally, there were no differences in raw fillet pH, L*, or b values. Male fillets were redder in color as indicated by a higher a-value. Females did produce firmer cooked fillets than males as measured by peak force and energy required to shear.

Fatty Acid Composition: Differences in the fatty acid composition of the fillets were primarily in the polyunsaturated fatty acid (PUFA) content (Table 3). Female fillets had higher amounts of 18:2n6, 18:3n3, 18:3n6, 20:2, 20:3n6, 20:5n3, 22:2, and 22:6n3. Conversely, male fillets had higher amounts of 20:4n6. Female fillets not only had higher amounts of PUFA, they specifically had higher amounts of omega 3 (ω3) and omega 6 (ω6) fatty acids when compared to their male counterparts. Fillet fatty acid composition reported as percent fatty acid is provided in Appendix 5, Table 7.

Gene Expression

Significance and n-values for all targeted genes expressed in the liver, muscle, and visceral adipose tissue are reported in Table 4.

Liver Gene Expression: Male liver had increased expression of lipogenic genes *gpat* and *scd1* (Figure 1a). The β-oxidation gene *ehhadh* was higher in male liver while *cpt1a* had higher expression in female liver (Figure 1b). Male liver had a two-fold increase in expression of fatty acid transport genes *lpl* and *me* (Figures 1c). Male liver also had higher expression of *cpt1b*, *mo25, rictor, pras40,* and *pparγ* (Figure 1d).

White Muscle Gene Expression: Lipogenic genes, *gpat, srebp1,* and *scd1* (Figure 2a), and fatty acid transporter, *cd36* (Figure 2c)*,* had higher expression in female white muscle when compared to male muscle. Female white muscle also had an increased expression of β-oxidation genes, *cpt1d* and *cpt2,* when compared to the male counterparts (Figure 2b). Female muscle had higher expression of *mo25,* and *raptor,* within the mTOR signaling pathway, than male muscle (Figure 2d).

Visceral Adipose Tissue Gene Expression: There were no significant effects of gender on the gene expression profile of the visceral adipose tissue (Figure 3; $P \geq 0.05$). However, there was a trend for males to have higher *cd36* expression than female visceral adipose tissue (Figure 3c; $P \leq 0.10$).

DISCUSSION

The objectives of the current study were to describe gender differences in growth and fillet quality and to identify differentially regulated genes as an indication of physiological mechanisms leading to these phenotypic differences. This study examined growth, fillet yield, composition, instrumental texture, fillet quality, and the associated fatty acid metabolism gene expression of liver, white muscle, and visceral adipose tissue. This experiment allowed for comparisons between immature male and female rainbow trout at roughly market size (1 kg at 14 M) and reduced environmental effects because fish were raised in communal tanks. Although there were no differences in growth between genders, there were distinct differences in the quality and compositional attributes investigated in this study. Additionally, these findings were

associated with differential gene expression in liver and white muscle. These findings suggest that immature, male and female rainbow trout have differing fatty acid metabolisms prior to the onset of sexual maturity and these differences may contribute to differences in fillet yield and quality.

Previous studies investigating weights and growth rates in sexually immature fish also report similar growth performance in male and female fish (Satue and Lopez, 1996; Memis and Gun, 2004; Batista-Pinto et al., 2009; Hale et al., 2011; Harmon, et al., 2011). Acharya (2011) found that immature female salmon have higher fillet yields than males; which supports our findings that female trout have higher percent separable muscle (1.5% greater) than their male counterparts. However, Acharya (2011) reported higher fat content in male salmon and firmer fillets in female salmon, as measured by breaking force. Conversely, we report no significant gender differences in fillet fat content, although males did have numerically higher amount of fillet fat. We also found firmer fillets from females as measured by the peak force and energy required to shear. There were discrepancies in husbandry practices, with salmon being raised in seawater net pens off the coast of Norway while the trout were raised in freshwater, partially-recirculated tanks under artificial, ambient photoperiod. Additionally, the salmon were over 2 years old when processed and the trout were roughly 14 M old at sampling. These differences in the studies did not appear to affect the growth or composition of the fish.

Although there were no differences in fillet crude fat content in our studies, there were gender effects on fatty acid profiles of the fillets. Female fillets had higher amounts of PUFAs, especially the ω3 and ω6 fatty acids. Salmonids, such as salmon and trout, differ in growth of maturing males and females, and these fish experience lipid mobilization and fillet quality deterioration during sexual maturation. However, these differences were not observed in the current study; primarily because fish were sampled prior to the onset of secondary sex characteristics (Nassour and Legar, 1989; Kiessling et al., 2001; Memis and Gun, 2004; and Manor et al., 2012).

No differences in growth and fillet proximate composition would suggest that underlying mechanisms regulating nutrient metabolism are similar for male and female fish, while

differences in fillet PUFA content suggests there may be differences in fatty acid metabolism. There are distinct expression profiles of genes involved in fatty acid metabolism in liver and white muscle of male and female rainbow trout. The presence of differentially regulated genes in these tissues reflects physiological mechanisms affected by gender in rainbow trout that may contribute to the observed differences in separable muscle and fatty acid profile. Male livers have an increased expression of *rictor* and *pras40.* Rictor is a cofactor involved in the assembly of TORC2 while PRAS40 inhibits TORC1 assembly (Laplante and Sabatini, 2011). There has been some evidence in lower organisms that suggest TORC2 acts as a negative regulator of lipid deposition in *rictor* null worms (Jones et al., 2009). However, male trout liver still had an up-regulation of *gpat* and *scd1,* which suggests increased levels of fatty acid synthesis. These contradictions are plausible as there is extensive post-transcriptional regulation of the mTOR signaling pathway (Lapanate and Sabatini, 2009; Caron et al., 2010; Laplante and Sabatini, 2011). Additionally, male livers had an increased expression of *pparγ,* which also up-regulates *scd1*.

Likewise, female white muscle had increased expression of fatty acid synthesis genes, *gpat, srebp1,* and *scd1,* along with increased expression of the mTOR cofactor *raptor.* The increased expression of these lipogenic genes may contribute to the increased PUFA content of female fillets. However, the physiological relevance of increased lipogenic genes in muscle is unclear as fish muscle demonstrates only very low levels of fatty acid synthesis (Rollin et al., 2003). Nevertheless, data suggest that the increased PUFA content of female fillets may result from increased lipogenic gene expression within white muscle. Gene expression data in the current study also suggest that females have increased capacity for β-oxidation, as indicated by increased expression of *cpt1a* in liver and *cpt1d* and *cpt2* in white muscle. Increased β-oxidation is associated with increased expression of TORC1 inhibitors *pras40* and *mo25* in liver and white muscle, respectively. However, extensive post-translational regulation of both the mTOR and PPAR signaling pathways plays a significant role in the activation of these pathways, which is not reflected in our measure of transcript abundance. Females having higher expression levels of genes involved in both fatty acid synthesis and β-oxidation suggest they may have higher fatty acid turnover than their male counterparts.

The PPAR signaling pathway, specifically PPARγ signaling, appears to be an important regulatory pathway in liver lipogenic gene expression. Male trout liver has an increased expression of *pparγ* and its target genes involved in fatty acid transport, *lpl* and *me.* Phenotypic data does not support increased lipogeneis in male trout because there were no differences in fillet crude fat; however males did have a numerically higher amount of fillet crude fat. In addition, there is no evidence of altered PPAR signaling in male and female white muscle or visceral adipose tissue. PPARs are widely studied in mammals, but data on fish PPARs is limited. In brown trout, PPARα is highly expressed in white muscle, heart, and liver, *pparβ* predominates in testis, heart, liver, white muscle, and trunk kidney, and *pparγ* was only quantified in the trunk kidney and liver (Batista-Pinto et al., 2005). Our study agrees with Ruyter et al. (1997) who determined a more diverse expression of *pparγ* in salmonids, where it is found not only in adipose tissue, but is also highly expressed in liver.

Expression of PPARs in brown trout is affected by gender and stage of development, with estrogen being a biological factor regulating expression of PPAR genes (Batista-Pinto et al., 2009). Estrogen elicits its effects on lipid metabolism through the estrogen receptor α (ERα) (Wend et al., 2013). Estrogen administration decreases adipocyte size and number in cultured mouse adipocytes by inducing lipolysis (Wend et al., 2013). Expression of *pparα* differed between genders only during early vitellogenesis (Batista-Pinto et al., 2009). Fish in the current study were sexually immature and harvested prior to the onset of vitellogenesis; therefore, the similar levels of *ppara* are in agreement with Batista-Pinto et al. (2009). Our data did differ from those of Batista-Pinto et al. (2009) in that we did not observe increased expression of *pparβ* in males pre-spawning compared to their female counterparts. Interestingly, our data did show decreased expression of *pparγ* in female liver. Differences in our findings compared to Batista-Pinto et al. (2009) could be the result of comparing 14 M old, first spawning rainbow trout to 3-yr old second spawning brown trout.

Ibabe et al. (2005) further investigated the role of estrogen (17β-estradiol) in *ppar* expression in isolated zebrafish hepatocytes and found that estrogen did not alter the expression of *pparα*, but 10μM estrogen decreased *pparγ* expression. The lack of differences in male and female *pparα* expression and the lower expression level of *pparγ* in female trout support these

findings. However, there is evidence that estrogen up-regulates *pparα* expression in rat hepatocytes (Campbell et al., 2003) and down-regulates *pparγ* in human bone marrow stromal cells (Heim et al., 2004). These results suggest effects of estrogen vary considerably with specie, tissue, and concentration used in cell culture and warrants more in-depth study, especially in fish (Ibabe et al., 2005). Hormones, such as estrogen, are significantly involved in regulating sexual maturation and, therefore, may also be involved in regulating gene expression within fatty acid metabolism and the mTOR and PPAR signaling pathways during this time period. This study indicates there are differences in the expression of genes involved in these pathways during immaturity when comparing male and female rainbow trout.

CONCLUSION

Data from this study indicate that gender affects nutrient regulatory mechanisms in rainbow trout prior to maturity. Although there were no differences in growth or fillet proximate composition between genders, there were distinct differences in the quality attributes investigated in this study. Females produced firmer fillets and had greater separable muscle than male trout. Additionally, female fillets had higher amounts of PUFAs, especially ω3 and ω6 fatty acids. Gene expression data suggest different capacities for fatty acid synthesis and β-oxidation in male and female trout, possibly leading to altered nutrient utilization. Specifically, increased expression of fatty acid synthesis and β-oxidation genes may contribute to the increased PUFA content of female fillets. Overall, these findings suggest immature male and female rainbow trout have differing fatty acid metabolism gene expression profiles prior to the onset of sexual maturity, primarily associated with the altered gene expression in the mTOR and PPARγ signaling pathways in liver and white muscle. These differences in gene expression may contribute to variation in product yield and quality between genders. Our findings suggest that gender-specific feeding strategies or diet formulations may maximize growth potential for single sex rearing; which is valuable as rainbow trout are often produced as all-female populations.

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TABLES and FIGURES

	Females	Males	p-value
WBW (g)	1022±48	993±65	0.0656
GSI (%)	0.30±0.03	0.34±0.13	0.7969
HSI (%)	0.97±0.02	1.04±0.04	0.1538
GtSI (%)	8.2±0.3	7.7±0.3	0.1950
TGC	2.1±0.1	2.0±0.2	0.2877
Feed Efficiency	0.86±0.32	0.78±0.14	0.9065
n	16	16	

TABLE 1: GROWTH PARAMETERS OF IMMATURE MALE AND FEMALE RAINBOW TROUT

Values are LSmeans ± SEM. Means without a common letter represents significant differences among the four phenotypic groups ($P \leq 0.05$). Abbreviations: WBW-whole body weight; GSI-gonadosomatic index; HSI-hepatosomatic index; GtSI-gastrointestinal tract; TGC-thermal growth coefficient.

	Females	Males	p-value
Yields			
Separable Muscle (%)	47.5±0.5[b]	46.3±0.7[a]	**0.0493**
Trim Loss (%)	29.9±0.4	30.1±0.9	0.6828
Fillet Thickness (mm)	21.6±0.5	20.5±1.1	0.0508
Belly Flap Thickness (mm)	10.3±0.4	9.8±0.6	0.3645
Cook Loss (%)	13.8±0.9	12.3±1.0	0.3638
pH	6.4±0.2	6.4±0.02	0.7741
Proximate Composition			
Moisture (%)	70.6±0.4	70.6±0.5	0.9622
Crude Fat (%)	8.6±0.4	8.9±0.5	0.2540
Crude Protein (%)	20.6±0.1	20.2±0.2	0.2023
Ash (%)	1.4±0.03	1.4±0.02	0.2936
Texture			
Peak Force (g force/g sample)	463±22[b]	385±26[a]	0.0162
Energy (kg/mm)	174.9±11.9[b]	149.9±13.7[a]	0.0483
Color			
L*	43.9±0.74	45.6±0.5	0.1723
a	1.3±0.1[a]	1.9±0.2[b]	**0.0385**
b	4.5±0.4	5.1±0.4	0.4541
n	16	16	

TABLE 2: FILLET CHARACTERISTICS OF IMMATURE MALE AND FEMALE RAINBOW TROUT

Values are LSmeans ± SEM. Means without a common letter represents significant differences among the four phenotypic groups ($P \leq 0.05$).

mg/100g	Female	Male	p-value
12:0	0.4±0.1	0.3±0.1	0.0949
13:0	0.1±0.01	0.08±0.01	0.2578
14:0	20.3±1.6	15.9±1.6	0.0670
14:1	0.6±0.1	0.5±0.1	0.0516
15:0	1.5±0.1	1.2±0.1	0.0744
16:0	128.2±10.6	106.4±9.8	0.1296
16:1	48.1±3.7	37.7±3.7	0.0582
17:0	1.2±0.1	1.0±0.1	0.2329
17:1	0.6±0.1	0.5±0.1	0.8655
18:0	25.4±2.1	23.2±2.1	0.4669
18:1n9	145.9±10.4	122.7±10.4	0.1292
18:2n6	91.0±6.3	63.1±6.3	**0.0047**
18:3n3	8.6±0.6	5.6±0.6	**0.0020**
18:3n6	1.5±0.1	1.0±0.1	**0.0092**
20:0	0.5±0.1	0.5±0.1	0.9818
20:1	10.5±0.8	9.3±0.8	0.2908
20:2	9.8±0.7	6.7±0.7	**0.0036**
20:3n3	0.5±0.04	0.4±0.04	0.0816
20:3n6	3.6±0.3	2.6±0.3	**0.0113**
20:4n6	0.3±0.3	1.6±0.2	**0.0391**
20:5n3	17.3±1.2	11.5±1.2	**0.0022**
21:0	0.2±0.02	0.1±0.02	0.3803
22:0	0.2±0.04	0.2±0.03	0.7247
22:1n9	1.2±0.1	1.2±0.1	0.9422
22:2	4.2±0.3	2.7±0.3	**0.0017**
22:6n3	56.0±4.2	39.3±4.2	**0.0100**
24:1	0.9±0.1	1.0±0.1	0.4509
SFA	177.9±13.7	148.9±13.7	0.1489
MUFA	207.9±14.9	172.9±14.9	0.1107
PUFA	187.2±12.5	129.8±12.5	**0.0035**
ω3	82.5±5.9	56.8±5.9	**0.0051**
ω6	118.8±8.2	82.3±8.2	**0.0044**
ω3: ω6	0.69±0.06	0.66±0.06	0.7248

TABLE 3: FILLET FATTY ACID COMPOSITIONS OF IMMATURE MALE AND FEMALE RAINBOW TROUT

Values are LSmeans ± SEM reported as mg of fatty acid per 100 g of sample. Means without a common letter represents significant differences between 14-month male and female rainbow trout ($P \leq 0.05$).

Gene	Liver	n (32)	Muscle	n (32)	Visceral AT	n (31)
Fatty Acid Synthesis						
gpat	**0.0130**	32	**0.0178**	32	0.8573	31
srebp1	0.3813	30	**0.0336**	25	ND	0
acyl	0.4420	27	0.3418	31	ND	0
acc	0.4471	32	0.7842	32	0.9712	31
fas	0.6157	32	0.9937	32	0.2819	31
scd1	**0.0461**	31	**0.0445**	32	ND	0
β-Oxidation						
magl	0.9315	29	0.7011	29	0.3639	31
cpt1a	**0.0034**	32	0.6798	32	0.2632	30
cpt1b	**0.0382**	23	0.2837	32	0.4080	31
cpt1c	0.3035	32	0.1649	31	0.1558	31
cpt1d	0.6117	21	**0.0044**	32	0.6489	31
cpt2	0.2379	30	**0.0244**	32	ND	0
aco	0.5880	32	0.4735	32	0.4063	31
acdhvl	0.4926	32	0.5261	31	0.5451	31
ehhadh	**0.0506**	32	0.5675	32	0.6605	31
acat2	0.1068	30	0.6462	31	0.6465	31
Fatty Acid Transport						
fabp3	0.6311	31	0.3650	32	ND	0
fabp4	0.1276	28	0.5979	32	0.9926	31
lpl	**0.0019**	31	ND	6	0.9197	30
cd36	0.6637	32	**0.0455**	31	0.0563	30
me	**0.0171**	32	0.8103	32	ND	0
Transcription Factors						
erk	0.8671	26	0.3191	32	ND	0
akt2	ND	0	0.6485	24	0.3455	31
redd1	0.8608	32	ND	8	0.7592	31
mo25	**0.0503**	32	**0.0504**	32	0.2649	31
mtor	0.3381	32	0.8196	32	0.1491	31
raptor	0.1334	31	**0.0471**	32	ND	0
rictor	**0.0220**	32	0.7289	32	0.6703	31
pras40	**0.0181**	32	0.2954	23	0.9718	31
pparα	ND	0	0.1901	31	ND	0
pparβ	0.9175	32	ND	1	0.8350	31
pparγ	**0.0139**	32	0.9897	32	0.7555	30
rxr	0.1964	30	0.2469	32	0.3793	31

TABLE 4: SIGNIFICANCE AND N-VALUES OF ALL GENES TARGETED BY THE MULTIPLEX IN LIVER, WHITE MUSCLE, AND VISCERAL ADIPOSE TISSUE OF IMMATURE MALE AND FEMALE RAINBOW TROUT

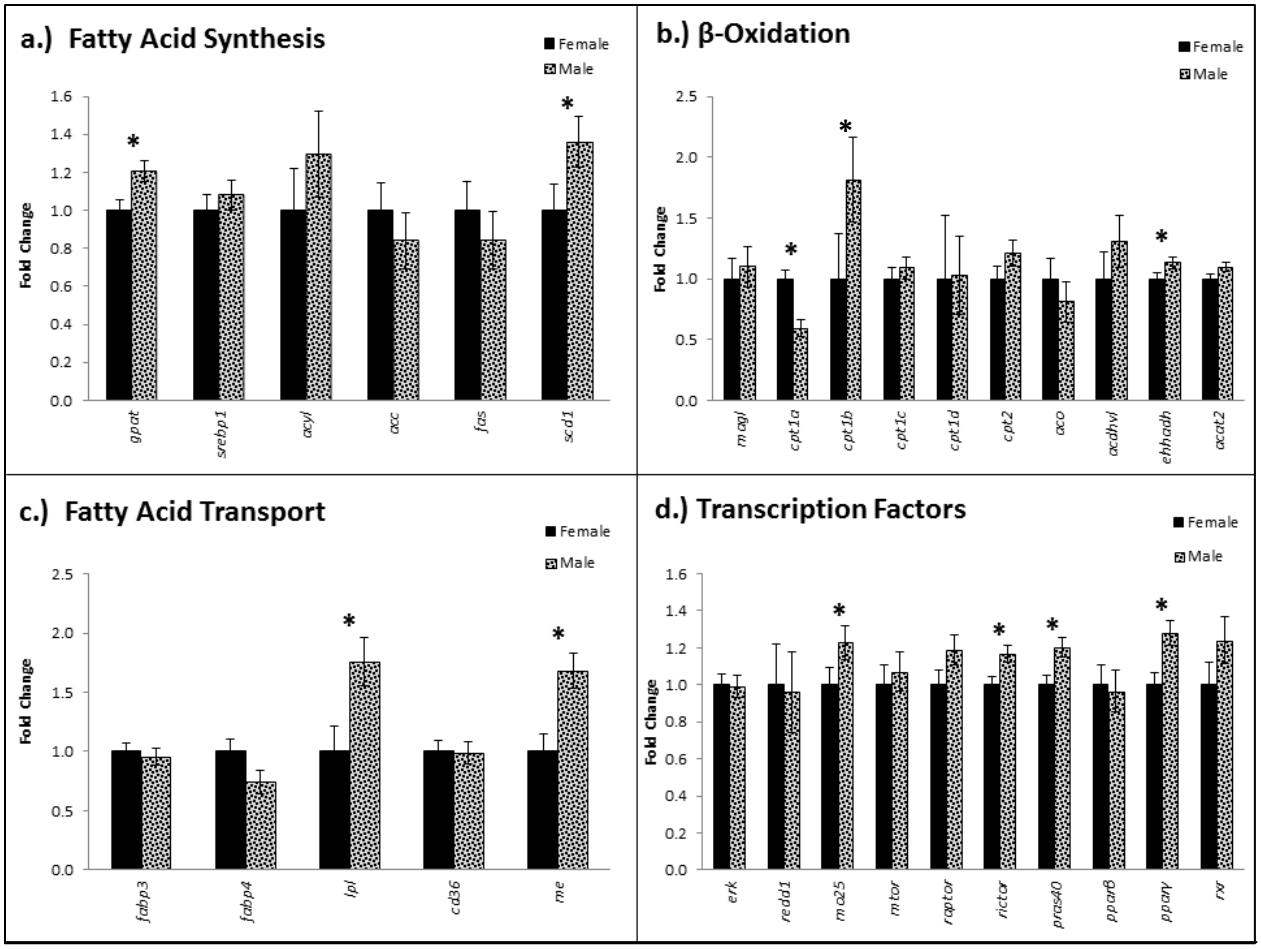

FIGURE 1: LIVER GENE RXPRESSION IN IMMATURE MALE AND FEMALE RAINBOW TROUT

a) Gender effects on genes within the fatty acid synthesis pathway expressed in the liver; b) Gender effects on genes within the β-oxidation pathway expressed in the liver; c) Gender effects on genes within the fatty acid transport expressed in the liver; d) Gender effects on genes of transcription factors with mTOR and PPAR signaling pathways. Values are LSmeans ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (β-*actin, eF1a, and rplp2)*. Significant differences between genders are indicated by asterisks ($P \leq 0.05$).

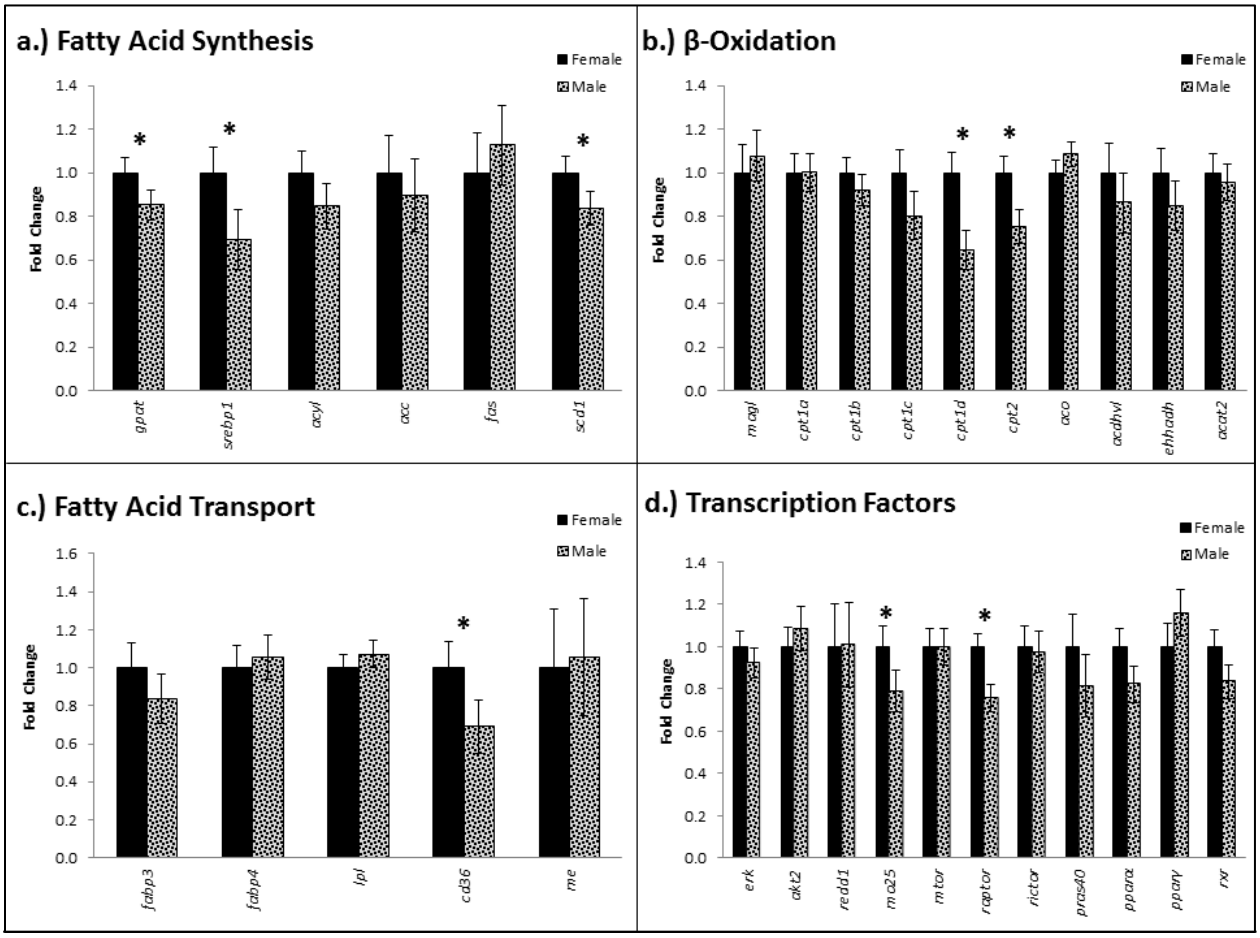

FIGURE 2: WHITE MUSCLE GENE EXPRESSION IN IMMATURE MALE AND FEMALE RAINBOW TROUT

a) Gender effects on genes within the fatty acid synthesis pathway expressed in the liver; b) Gender effects on genes within the β-oxidation pathway expressed in the liver; c) Gender effects on genes within the fatty acid transport expressed in the liver; d) Gender effects on genes of transcription factors with mTOR and PPAR signaling pathways. Values are LSmeans ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (β-*actin, eF1a, and rplp2)*. Significant differences between genders are indicated by asterisks ($P \leq 0.05$).

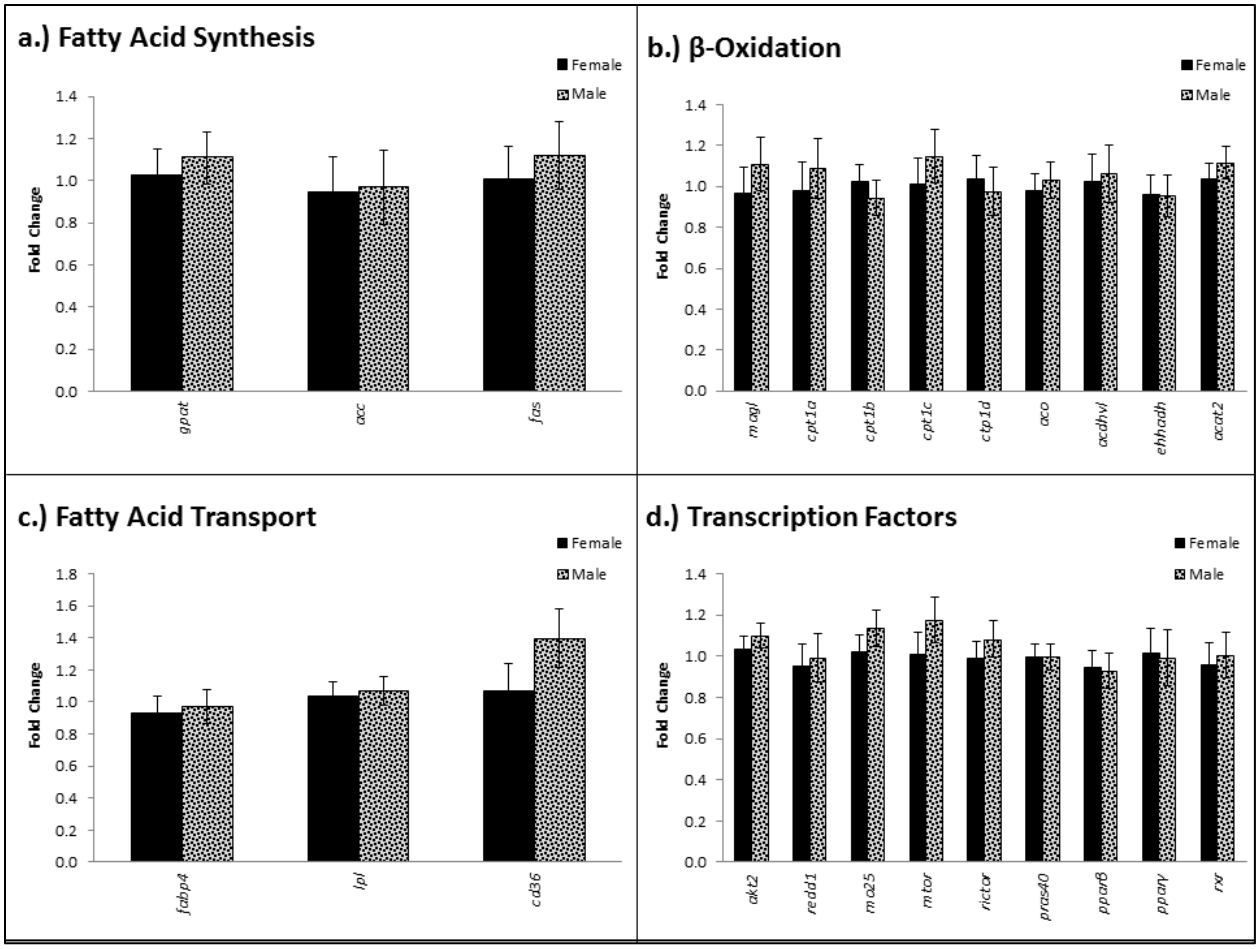

FIGURE 3: VISCERAL ADIPOSE TISSUE GENE EXPRESSION IN IMMATURE MALE AND FEMALE RAINBOW TROUT

a) Gender effects on genes within the fatty acid synthesis pathway expressed in the liver; b) Gender effects on genes within the β-oxidation pathway expressed in the liver; c) Gender effects on genes within the fatty acid transport expressed in the liver; d) Gender effects on genes of transcription factors with mTOR and PPAR signaling pathways. Values are LSmeans ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (β -*actin, eF1a, and rplp2)*. Significant differences between genders are indicated by asterisks ($P \leq 0.05$).

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CHAPTER 5:

Title

Associations of fillet yield and fat content with fillet texture, fatty acid composition, and fatty acid metabolism gene expression in female rainbow trout

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Keywords

Salmonid, GeXP-Multiplex, mTOR, PPAR, lipid

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ABSTRACT

To determine the associations of fillet yield and fat content on fillet quality attributes and gene expression, 14-month old female rainbow trout were chosen, after processing, based on fillet yield and crude fat content. Families were divided into four phenotypic categories: low yield/low fat (LY/LF; n=22), low yield/high fat (LY/HF; n=22), high yield/low fat (HY/LF; n=24), and high yield/high fat (HY/HF; n=24). LY/LF fish had the lightest whole body weight, and HY/HF fish were heaviest. The only difference in fillet fatty acid composition was that HF groups contained higher amounts of monounsaturated fatty acids than LF groups. LF groups had higher hepatic expression of *scd1* compared to HF groups. LY/LF muscle had increased *cpt1d* expression that may contribute to a lower fat content since this is the rate-limiting gene for β-oxidation. In visceral adipose tissue, *akt2* was expressed more in HF groups compared to LF groups. The correlations of β-oxidation genes, specifically *cpt1* isoforms, in white muscle with fillet fat content and shear force values suggests increased β-oxidation is a mechanism negatively affecting fillet fat content and quality by decreasing the amount of lipid within the muscle and altering the firmness of the fillet. Overall, data suggest that differences in growth and fillet quality phenotypes may result from variation in the capacity for β-oxidation; fat content may be associated with the mTOR signaling pathway and yield with the PPAR signaling pathway.

INTRODUCTION

The aquaculture industry has improved the cultivation of salmonids through various approaches, that include establishing breeding programs, optimizing feeds, improving disease treatments, and reducing production times; however, low slaughter yields and less visceral fat content are areas of potential improvement (Rasmussen and Ostenfeld, 2000). Improving these areas of production will aid in enhancing the overall quality of fish products. Fillet quality is impacted by a diverse set of physical, microbiological, and nutritional attributes that vary among market sectors, regions, cultures, and individuals (Setala et al., 2000). Nutritional value, flawlessness, and firmness of fillets are high-ranking quality attributes to consumers (Setala et

al., 2000; Rasmussen, 2001). The quality of the raw fillet, including the cultivation environment, size, fat content, and the overall product consistency, has the greatest value to producers (Setala et al., 2000). Fauconneau et al. (1995) suggest that maximum protein and lipid levels of fish fillets should be reached to achieve higher quality products. However, it is not simply the lipid content of the fillet; it is the lipid composition that is of primary importance when considering the nutritional value of fillets. Salmonids, such as salmon and trout, are known for producing fillets with high amounts of polyunsaturated fatty acids (PUFA), especially the omega-3 fatty acids (ω3). These fatty acids are important to human nutrition because of their implications in cardiovascular and neural health (Spector, 1999). Therefore, increasing the amount of fat, specifically PUFA, in the muscle, while reducing the amount of fat discarded as visceral waste, will increase fillet yield and generate a higher quality product for producers and consumers.

Although lipid content of fillets is regularly manipulated by diet, little is understood about the regulation of lipid deposition within the various adipose tissue depots. Two pathways known to regulate fatty acid metabolism are the mechanistic target of rapamycin (mTOR) and peroxisome proliferator activated receptors (PPAR) pathways (Laplante and Sabatini, 2011; Poulsen et al., 2012). Understanding how genes within these two pathways are regulated and their associations with phenotypic traits will improve our ability to develop better management and breeding practices for more efficient food-fish production.

The mTOR pathway is a central signaling cascade that plays a role in integrating energy-sensing pathways. The mTOR signaling pathway in fish is less characterized than that of mammals; however, the consensus is that the mTOR signaling pathway is highly conserved among species based on limited *in vitro* and *in vivo* studies (Plagnes-Juan et al., 2008; Seiliez et al., 2008; Lansard et al., 2009; Lansard et al., 2010; Seiliez et al., 2011). Most studies investigating mTOR in salmonids have focused on effects of insulin (Plagnes-Juan et al., 2008; Lansard et al., 2010) or feeding regimen (Lansard et al., 2009; Seiliez et al., 2010) on energy, mostly protein metabolism. There was some assessment of the fatty acid synthesis pathway by investigating expression of *fas, srebp1,* and *cpt1* genes using trout divergently selected for high and low muscle fat for three generations to determine if there was an altered nutrient utilization through changes in the mTOR signaling pathway (Skiba-Cassy et al., 2009). Skiba-Cassy et al.

(2009) concluded that genetic selection for increased muscle fat content results in over activation of the mTOR signaling pathway and increased expression of lipogenic genes in rainbow trout.

The PPAR signaling pathway is known to respond to lipids and elicit transcriptional changes of genes involved in fatty acid metabolism in mammals. PPARs are widely studied in mammals, but little is known about PPARs in salmonids. Most studies involving PPARs in salmonids characterize gene expression in various tissues (Ruyter et al., 1997; Andersen et al., 2000; Batista-Pinto et al., 2005). Gender and developmental stage are known to influence the expression of all PPARs in brown trout liver (Batista-Pinto et al., 2009). Nevertheless, few studies have investigated the relationship between gene expression within fatty acid metabolism and specific phenotypes of salmonids (Kolditz et al., 2008; Skiba-Cassy et al., 2009; Kolditz et al., 2010). This study uniquely investigated specific associations between gene expression of major lipid depots (liver, muscle, and visceral adipose tissue) and fillet quality attributes (yield and composition) with a focus on fatty acid synthesis and β-oxidation pathways.

The objective of this study was to determine the association of variations in fillet yield and fat content with fillet quality parameters and gene expression in rainbow trout. Full-sib families originating from the National Center for Cool and Cold Water Aquaculture (NCCCWA; U.S. Department of Agriculture, Agricultural Research Service. Keraneysville, WV, U.S.A.) selective breeding program were divided into four phenotypic categories according to family mean fillet yield and fat content: low yield/low fat (LY/LF), low yield/high fat (LY/HF), high yield/low fat (HY/LF), and high yield/high fat (HY/HF). Aspects of fillet quality investigated in this study included proximate composition, instrumental texture, color, and fatty acid content. In addition, the relative expression levels of 35 genes were analyzed to determine if the physiological basis for the phenotypes can be attributed to differential regulation of gene expression within fatty acid metabolism. Furthermore, this study characterized quality of rainbow trout from an intensive breeding program focused on improving growth rate. It is hypothesized that muscle yield and fat content will be associated with altered fillet quality attributes and expression of genes involved in regulating fatty acid metabolism.

MATERIALS and METHODS

Experimental Design

Ninety-eight rainbow trout families from the NCCCWA population selected for three generations for greater whole body weight (WBW) and thermal growth coefficient (TGC) at 10 months of age were evaluated for fillet yield and fillet fat content. All fish were hatched within a three-week period in late March/early April of 2010 and reared at the NCCCWA according to standard operating procedures. Passive integrated transponders (PIT-tags; Avid Identification Systems Inc., Norco, CA, U.S.A.) were inserted in the left dorsal musculature at approximately 5.5 months of age (~75 g WBW) in 17 fish per family for individual identification. At approximately 14 months of age, five fish were systematically sampled from each family to capture within-family variation for WBW (e.g., the 3rd, 6th, 9th, 12th, and 15th largest fish per family). Fish within a family were randomly assigned to one of five harvest groups, and one group per week was harvested over a five-week period. Gravimetric measurements, fillet yield, and proximate composition analysis were completed on all fish (490 fish). For the current study, a subset of families were selected based on separable muscle [high yield—HY (52-49%) and low yield—LY (44-47%)] and fillet crude fat content [high fat—HF (9-11%) and low fat—LF (6-7%)]. Five families per phenotype combination were selected (LY/LF, LY/HF, HY/LF, and HY/HF) for a total of twenty families. Family selection was dependent on the family having at least five female fish; therefore there was a total of 20 fish within each phenotypic group. Fillet fatty acid profiles and gene expression were subsequently analyzed on these twenty families only.

Animal Husbandry

Methods for animal husbandry followed the guidelines outlined by the US Department of Agriculture (USDA) and NCCCWA Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals.* Fish were reared in flow-through spring water for the first three months and then partially-recirculated spring water thereafter. Water temperatures ranged from 12.4 to 14.0^{o}C. Between hatching and tagging, fish were reared separately by family in 200-L tanks, and then co-mingled in 1,000-L tanks after tagging for growth performance evaluation until approximately 13 months of age. At

approximately 8 and 10 months of age, fish were weighed and split into additional 1,000-L tanks to maintain biomass densities ≤80 kg/m^3. Fish were fed a commercial fishmeal-based diet consisting of 16% crude fat and 42% crude protein (Ziegler Bros. Inc., Gardners, PA, U.S.A.) using automated feeders (Arvotec, Huutokoski, Finland) with a feeding schedule similar to that given in Hinshaw (1999). At the end of the growth performance evaluation period, fish were weighed and separated by harvest group into five 1,000-L tanks (98 fish per tank), with one fish per family per tank. Feed was withheld five days prior to harvest. Fish were harvested using an overdose of tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, CA, U.S.A.), and WBW and weights of gonads, liver, and gastrointestinal tract were recorded. Weights were used to calculate the gonadosomatic index (GSI), hepatosomatic index (HSI), and gastrointestinal tract somatic index (GtSI), respectively; tissue weights were expressed as a percent of WBW. Subsamples of epaxial muscle, liver, and visceral adipose tissue were taken, immediately frozen in liquid nitrogen, and stored at -80°C for gene expression analysis. Carcasses were packed on ice and processed the following day at the West Virginia University Muscle Foods Laboratory (Morgantown, WV, U.S.A.). Boneless, skinless fillets were removed from each fish, weighed, and analyzed for color. The remainder of the fillet, after sectioning for texture analysis as described below, was vacuumed-packaged and stored at -20°C until powdering for compositional analyses.

Thermal Growth Coefficient and Feed Efficiency

Thermal growth coefficient (TGC) was calculated for each fish as $[(\sqrt[3]{W_f} - \sqrt[3]{W_i})/(T \times t)] \times 1{,}000$, where W_f and W_i are 13-month WBW and 10-month WBW, respectively, T is water temperature (°C), and *t* is time in days (Iwama & Tautz, 1981; Jobling, 2003). A constant 13°C was assumed for all TGC calculations because all tanks were supplied with water from the same source and water temperature did not vary substantially during this growth period.

Feed intake was measured in triplicate on individual fish over a two-week period at 12.8 months of age (mean body weight = 860 grams) as a means to estimate feed efficiency. Un-pelleted meal from the commercial diet was sampled from the manufacturer, labeled with approximately 0.2% w/w of 0.4-0.6 mm diameter leaded-glass ballotini beads (Sigmund Linder GmbH, Warmensteinach, Germany), and pelleted and oil coated at the Bozeman Fish

Technology Center (Bozeman, MT, U.S.A.) in a manner similar to that used to manufacture the unlabeled commercial diet. On each of three days, occurring one week apart, fish were fed the labeled feed using the same automated feeders at the same feeding rate as the normal diet. The labeled feed was fed for the first half of the day (i.e., 3.5 hours), after which feeding stopped and the fish were weighed and imaged using x-ray. The x-ray opaque ballotini beads were counted from each radiograph and used to estimate feed intake. Feed efficiency was estimated as gain in body weight ÷ weight of feed consumed, whereby gain in body weight represents total body weight gain during the two-week period and weight of feed consumed was calculated as the average of three repeated intake measurements and extrapolated over the two-week period.

Fillet Characteristics

Fillet Yields: Separable muscle is reported as a percent of WBW. Trim included the head, vertebral column, ribs, belly flap, and skin and is reported as a percent of WBW. Fillet thickness for each fish was measured using calipers at the thickest part of the fillet.

Quality Attributes: Fresh fillet surface color was recorded with a chromameter (Minolta, Model CR-300; Minolta Camera Co., Osaka, Japan). This instrument was color calibrated using a standard white plate No. 21333180 (CIE Y 93.1; x 0.3161; y 0.3326), and L* (lightness), a* (redness), and b* (yellowness) values were recorded at the cranial, middle, and caudal regions of the fillet.

Texture analysis was performed on 8x4-cm fillet sections that were taken, centered on the lateral line, 2–3 cm caudal to the pectoral girdle. Sections were stored at 4^{o}C overnight on PVC-overwrapped trays. Sections were then thermally processed in a microprocessor-controlled smoke oven (Model CVU-490; Enviro-Pak, Clackamas, OR, U.S.A.) set at 82^{o}C, and the cooking process was stopped when the internal temperature reached 65.5^{o}C. This cooking temperature was selected according to the USDA recommended minimum internal temperature for fish to achieve a safe temperature without overcooking (Nilsson and Ekstrand, 1995). Total cooking time was approximately 45 min. After cooking, product was allowed to cool to room temperature. Weights of the raw and cooked sections were recorded to calculate percent cook loss. Instrumental texture was measured using a 5-blade, Allo-Kramer shear attachment mounted

to a TA-HDi® Texture Analyzer (Texture Technologies Corporation; Scarsdale, NY, U.S.A.). The texture analyzer was equipped with a 50-kg load cell and tests were performed at a crosshead speed of 127mm/min. Shear force was applied perpendicular to the long axis of the fillet. Force deformation curves were recorded, and maximum shear force (gram per gram of sample) and total energy of shear (grams/mm) were determined using the Texture Expert Exceed software (version 2.60; Stable Micro Systems Ltd., Surrey, UK).

Proximate Analyses: Boneless-skinless fillets were stored at -20^{o}C under vacuum for no more than one month before being snap-frozen in liquid nitrogen and powdered using a Waring commercial grade blender (Model 51BL31; Waring Commercial; Torrington, CT, U.S.A.). Powdered sample was and stored at -80°C until analysis. Moisture, crude lipid, crude protein, and ash content were determined using AOAC approved methods (AOAC, 2000). Moisture analysis was performed by weighing the sample before and after an 18 hr drying period at 110°C. Crude lipid content was determined indirectly using petroleum ether in a Soxhlet extractor. Crude protein was calculated by converting percent nitrogen (Kjeltec^{TM} 2300; Foss North America; Eden Prairie, MN, U.S.A.) to crude protein using 6.25 as the conversion factor. Ash was determined by incinerating samples at 550^{o}C in a type A1500 furnace (F-A1525M-1; Thermolyne Corporation; Dubuque, IA, U.S.A.).

Fatty Acid Analysis: Total lipids were extracted from 0.5-g samples of powdered muscle tissue, according to Bligh and Dyer (1959), using a chloroform-methanol mixture (4:1 v/v). Fatty acids were methylated using the method described by Fritshe and Johnston (1990). Nonadecanoic acid (19:0) was used as an internal standard. Fatty acid methyl esters (FAMEs) were quantified using a Varian CP-3800 Gas Chromatograph (Varian Analytical Instruments; Walnut Creek, CA, U.S.A.) equipped with a flame ionization detector. A wall-coated, open-tubular fused silica capillary column (100m length, 0.25mm inside diameter; Varian Inc., Walnut Creek, CA, U.S.A.) was used to separate FAMEs. The stationary phase was CP-Sil 88, and nitrogen was the carrier gas at a flow of 0.3mL/min. A 10:1 split ratio was applied for all samples. An oven temperature of 140^{o}C for 5 min, followed by a temperature ramp of 3^{o}C/min to 235^{o}C, was used; dwell time at 235^{o}C was 15 min. The total separation time per sample was 68.5 min. Injector (11-77 injector, Varian Inc., Walnut Creek, CA, U.S.A.) and detector (Flame

Ionization Detector-FID, Varian Inc., Walnut Creek, CA, U.S.A.) temperatures were maintained at 270^{o}C and 300^{o}C, respectively. FAMEs were identified based on comparison to retention times of standard FAMEs (SupelcoTM quantitative standard FAME 37; Sigma-Aldrich, St. Louis, MO, U.S.A.). Peak area counts were computed by an integrator using the Star GC workstation version 6 software (Varian Inc., Walnut Creek, CA, U.S.A.). Fatty acids were reported as mg/g of sample.

Gene Expression Analysis

Multiplex Analysis: The GenomeLab GeXP genetic analysis system (Beckman Coulter Inc.) was used to simultaneously analyze expression of thirty-nine genes in liver, white muscle, and visceral adipose tissue. Within the multiplex, thirty-five genes were associated with fatty acid metabolism and four served as potential reference genes. Primers were designed using eXpress Designer software (Beckman Counter Inc., Pasadena, CA, U.S.A.), and primer sequences were compared against other rainbow trout gene sequences using the BLAST function within the NCBI database to reduce unintended sequence amplification. The size of each amplicon was confirmed based on its expected length. No undetermined peaks interfered with amplification of the intended multiplex. Optimization of the multiplex, standard curves, reverse transcriptase (RT) and PCR reactions, and capillary electrophoresis were performed as recommended by the manufacturer (GeXP Chemistry protocol A29143AC; February, 2009) with reagents provided in the GeXP Start Kit (Beckman Coulter Inc., Pasadena, CA, U.S.A.). GenBank accession numbers, database reference numbers, and references for the sequences used to generate multiplex primers are shown in Appendix 4, Table 6. A summary of the associated regulatory pathway, role in lipid metabolism, and R^2 values for the RNA standard curve (0.2 ng /μL – 100 ng/μL) for each gene is also reported in Appendix 4, Table 6. Primer sequences that include universal tags are provided in Appendix 3, Table 5.

Areas for each peak within the multiplex were exported to eXpress Profiler software (Beckman Coulter, Inc., Pasadena, CA, U.S.A.) for analysis and normalization to the internal kanamycin control. Concentrations were interpolated from the standard curves for each gene of interest. GeNorm software was used to determine which reference genes were most stable. The most stable reference genes were *β-actin*, *rplp2*, and *ef1a* for all three tissues. M-values for these

three genes and for all three tissues were below 0.5; therefore, their geometric mean was used to generate a normalization factor for each sample. Thus, the normalized expression of each gene transcript is reported as the quantity relative to the geometric mean of the selected reference genes with arbituray units.

RNA Isolation: To isolate RNA, 50 – 100 mg of tissue was homogenized in 1 mL TRIzol (Invitrogen, Carlsbad, CA, U.S.A.) per manufacturer's suggested protocol using a 5 mm steel bead and a multi-tube shaker. RNA was isolated per manufacturer's protocol. The RNA pellet was washed twice with 75% ethanol, and re-suspended in nuclease-free water. RNA quality and quantity was determined by measuring absorbance at 260 nm and 280 nm.

Multiplex PCR: The multiplex reverse transcription (RT) reactions were optimized for each tissue. Reverse transcription reactions included 2 µL 5X RT buffer, 1 µL gene-specific reverse primer mix, 0.5 µL RT, and 1.25 µL kanamycin RNA (internal control) in a 10 µL reaction. Reactions included 1.25 µL, 2.5 µL, and2.5 µL of 100 ng DNase treated RNA for liver, white muscle, and visceral adipose tissue respectively. The RT was incubated according to kit instructions. An aliquot (4.65 µL) of the resultant cDNA was used in PCR reactions for all three tissues that included 2 µL 25 mM $MgCl_2$, 2 µL 5X PCR buffer, 1 µL forward primer mix, and 0.35 µL DNA Taq polymerase. The PCR was incubated according to kit instructions. 1 µL of PCR products was combined with 38.5 µl sample loading solution and 0.5 µL size standard 400. The PCR products were separated by capillary electrophoresis in the GeXP Genetic Analysis System using a modified Frag-3 protocol with a separation voltage of 6.0 kV for 45 min.

Statistical Analysis

Data were analyzed using analysis of variance to test for main effects of phenotype and family with PC-SAS (Version 9.1, 2004) general linear models procedure. A 4x5x5 (phenotype x family x tank) design was used to test main effects; a total of 92 fish were included in the experiment because of the female constraint. Effects were considered significant at $P \leq 0.05$. There were no significant effects of tank. Data are presented as LSMeans ± SEM (standard error of the mean). Correlations using individual fish data were analyzed with the PROC CORR procedure of PC-SAS (Version 9.1, 2004). A Bonferroni correction was used to reduce the

occurrence of Type I errors due to multiple comparisons; the corrected significance value was $P \leq 0.0018$.

RESULTS

Growth Responses

All growth responses are reported in Table 1. The LY/LF group had the lowest WBW (772±45g) and the HY/HF group had the largest WBW (1152±43g). Phenotype affected GSI ($P \leq 0.05$); however, as expected, gonads were small and underdeveloped as GSI averages ranged from 0.25±0.02% to 0.33±0.02%. Therefore, fish were in the very early stages of sexual maturation and were harvested prior to the point at which maturation-related processes affect growth performance and fillet attributes. HSI was highest for the LY/HF phenotypic group (1.20±0.03%) and lowest for the HY/LF phenotypic group (0.98±0.03%). GtSI was lowest for the HY/LF phenotypic group (7.04±0.31%) and highest for the LY/HF phenotypic group (9.33±0.32%). TGC was also affected by phenotype with the HY/HF phenotypic group having the highest TGC (2.3±0.05) and the LY/LF phenotypic group exhibiting the lowest TGC (1.9±0.06). Feed efficiency was not affected by phenotype ($P > 0.05$; Table 1).

Fillet Characteristics

All fillet quality attributes are reported in Table 2. Fillet yield, as measured by percent separable muscle, was one criterion used for categorizing fish for this study; therefore a significant effect of phenotype was expected. Yields were 46.0±0.04% and 47.1±0.04% for the LY groups and 49.8±0.04% and 50.3±0.04% for the HY groups. Low yield groups had higher trim losses than HY groups. In addition, LY groups had thinner fillets when compared to HY groups. The HY/HF phenotypic group had the lowest cook loss (12.6±0.3%) and the LY/LF phenotypic group had the highest cook loss (14.2±0.3%). The other parameter used to categorize fish was crude fat of the fillet, which, as expected, was affected by phenotype. Crude fat was 7.1±0.3% and 6.8±0.3% for the LF groups and 9.6±0.3% and 9.9±0.3% for the HF groups. Fillet moisture was inversely related to crude fat, as anticipated. There were no significant differences

in fillet protein content (P>0.05). LY phenotypic groups had higher percent ash than HY groups. The LY/LF phenotypic group had the lowest peak force and energy of shear while the HY/HF phenotypic group had the firmest fillets. LF raw fillets had lower L*-values and b-values than HF fillets. There were no significant effects of group on a-values for raw fillets.

Fillet Fatty Acids

The saturated fatty acids 12:0 and 20:0 were significantly higher in the HY/HF group while 14:0, 16:0, and 18:0 trended higher in the HF groups (P≤0.10) (Table 3). The monounsaturated fatty acids (MUFA), 14:1, 16:1, and 18:1, were also higher in HY/HF group, with 18:1 being 30% higher in the HY/HF group (6.63 ± 0.56mg/g) than in the HY/LF group (4.60 ± 0.56mg/g). Total MUFA was affected by phenotype with the HY/HF group having 2.9mg/g more MUFA than HY/LF group. Total SFA trended higher in the HF groups as well, (P≤0.10). There were no significant effects of phenotype on any of the PUFAs. Additionally, there were no significant effects of family on any of the fatty acids measured in this study. Percent fatty acid data are reported in Appendix 6, Table 8.

Gene Expression

Significance and n-values for all genes expressed in the liver, muscle, and visceral adipose tissue are reported in Table 4.

Liver: The only gene in the liver to be affected by phenotype was *scd1*; LF groups had higher expression of *scd1* compared to the HF groups (figure 1a). Additionally, there were significant positive correlations between muscle crude fat content and *fabp3* (p=0.0018; r^2=0.4277), *lpl* (p=0.0015; r^2=0.4912), and *scd1* (p=0.0015; r^2=0.4988).

White Muscle: Phenotype did not affect genes expressed in white muscle (P > 0.05); however, *cpt1d* and *magl* did show trends at P≤0.10 (figure 2). LY/LF fish had the highest expression of *cp1d* and the lowest expression of *magl*. LY/LF expression of *magl* was 85% lower than the LY/HF group (69.07 ± 102.55 and 467.01 ± 105.09 normalized transcript abundance (ng/µL), respectively). Additionally, force required to shear is positively correlated with the β-oxidation gene *cpt1a* (p=0.0012; r^2=0.5182).

Visceral Adipose Tissue: HF groups had greater expression of *akt2* compared to the LF groups (figure 3d). Whereas *rictor* and *gpat* had similar expression patterns, with the LY/HF and HY/LF groups having more transcripts than the LY/LF and HY/HF groups. The only gene in the visceral adipose tissue to show a trend ($P \leq 0.10$) was *scd1*. The LY/HF group had almost double the expression of the LY/LF group; 276.01 ± 37.23 and 151.09 ± 33.30 normalized transcript abundance (ng/μL), respectively (figure 1a). Additionally, GtSI was negatively correlated with β-oxidation genes, *cpt1c* ($p=0.0018$; $r^2=-0.3967$), *redd1* ($p=<0.0001$; $r^2=-0.5235$), and *pparb* ($p=0.0016$; $r^2=-0.4886$). Trim losses were positively correlated with redd1 ($p=0.0017$; $r^2=0.4373$) expression in visceral adipose tissue.

DISCUSSION

The objective of the current study was to evaluate potential molecular mechanisms leading to increased muscle yield and muscle fat accumulation in female rainbow trout at approximately market size (1 kg at 14 M). This study examined growth, fillet yield, composition, mechanical texture, quality, and the associated gene expression of liver, white muscle, and visceral adipose tissue. This approach allowed for comparisons between fish with high and low muscle yield and fat content that had been selected for increased growth for three generations. Environmental effects were reduced by rearing fish in communal tanks. Furthermore, fish were immature females, avoiding any effects of sex or sexual maturation. Although muscle yield and fat content differed only by approximately 3% for each variable, distinct associations were observed between fillet quality and gene expression data, suggesting that subtle genetic effects on physiological processes can impact phenotype and economics of production.

There is evidence that fast-growing strains of rainbow trout have increased adipose tissue accretion (Fauconneau et al., 1995). However, this research indicates that rainbow trout selected for growth vary in total lipid content and adipose tissue deposition. GtSI can serve as an index of the amount of visceral fat stores, and visceral adipose tissue associated with the digestive tract is

a major contributor to the weight of the viscera in rainbow trout (Regost et al., 2001). GtSI was variable among the phenotypic groups with the HY/LF group having the lowest GtSI and LY/HF group having the largest amount of visceral adipose tissue. This translated into the HY/LF group having low trim losses and the LY/LF group having the highest trim losses along with the greatest cook losses. Cook losses are associated with cooking rate and moisture and lipid lost during cooking, in turn affecting palatability. Interestingly, the fastest growing fish, HY/HF, as measured by the WBW and TGC, did not have the largest visceral adipose tissue stores. This finding is beneficial to the aquaculture industry since the aim of production is to reduce the percentage of offal (Rassmussen, 2001); this reduction was observed in this phenotypic group without compromising growth, decreasing fillet fat content, or fillet yield. These are unique findings since slaughter yield decreased with fish size in larger salmonids (Einen and Skrede, 1998), and fast-growing strains of rainbow trout exhibited lower carcass yields (Morkramer et al., 1985).

Several fillet quality attributes varied by phenotypic groups. LY/LF fish produced the softest cooked fillets, as measured by peak force and energy required to shear, and the darkest, most yellow raw fillets. However, crude protein content did not differ among the phenotypic groups supporting Shearer's (1994) conclusion that protein content is influenced by diet or genetics less than fillet lipid content. Since the diet used in this study did not differ among groups, few differences were observed in the fatty acid composition of the fillet. This consistency was expected because the primary contributor to muscle fatty acid composition is diet (Turchini and Francis, 2008). Primary differences were within the medium-chain MUFAs; HY/HF fillets had the highest amounts of 14:1, 16:1, and 18:1 fatty acids. In general, HY/LF fish had the most desirable fillet characteristics including bright, firm attributes that are preferred by consumers (Rasmussen, 2001), and high yields that are preferred by producers (Setala et al., 2000). Additionally, HY/LF fish had the highest ω3:ω6 ratio while maintaining optimal growth, supporting the Bugeon et al. (2010) assertion that increasing fillet yield does not negatively impact fillet quality. Our suggestion that HY/LF fish produce the highest quality fillets may contradict Fauconneau et al. (1995), who suggest that maximum protein and lipid levels should be reached to achieve higher quality products.

Nutritional manipulation of intramuscular fat, independent of other body fat stores, is difficult; therefore, biological markers for increased intramuscular fat would be beneficial, allowing for prediction of muscle adiposity at an early age. However, identification of biomarkers relies on existing scientific knowledge regarding regulation of intramuscular fat content by physiological and nutritional factors (Hocquette et al., 2010). Albeit, little is known about metabolic integration that regulates nutrient partitioning in fish. Two pathways that regulate nutrient metabolism in mammals are the mTOR and PPAR pathways (Laplante and Sabatini, 2011; Poulsen et al., 2012). Both pathways respond to nutrient availability and alter target gene expression of key enzymes involved in fatty acid metabolism. However, both pathways do not uniquely control lipid metabolism (Laplante and Sabatini, 2011; Poulsen et al., 2012). Several genes within both pathways have the potential to serve as markers for increased intramuscular fat; several of those potential genes are included in the multiplex used in this study (Hocoquette et al., 2010).

Skiba-Cassy et al. (2009) investigated the role mTOR plays in controlling lipid synthesis. These authors wanted to determine if divergent selection for high and low muscle fat altered nutrient utilization through changes in mTOR signaling in rainbow trout. Body weight and feed intake were not different between the two groups; this finding suggests there are metabolic differences in nutrient utilization between the lines resulting in the different phenotypes (Skiba-Cassy et al., 2009). Our study did not reveal differences in feed intake or feed efficiency. Moreover, Skiba-Cassy et al. (2009) determined that *mtor* transcripts were more abundant in the liver of the fat-line fish. However, our data did not reveal differences in *mtor* expression in any of the tissues measured; but, most of the genes investigated in this study are subject to posttranscriptional regulation.

In addition, Skiba-Cassy et al. (2009) reported low *cpt1* expression in the fat-line fish compared to the lean-line fish, suggesting a decreased ability for β-oxidation, which may contribute to accumulation of lipid stores. Our results support the concept that β-oxidation affects composition and yield because there was a trend for increased expression of *cpt1d* in white muscle of LY/LF fish ($P \leq 0.10$). Combining these findings and those of Corraze et al. (1999), who determined that *de novo* synthesized lipids are preferentially incorporated in muscle rather

than adipose tissue, Skiba-Cassy et al. (2009) concluded genetic selection for increased muscle fat content results in over activation of the mTOR signaling pathway and increased expression of lipogenic genes. Although our study used fish selected on growth rather than muscle fat content, our fish do have variation in muscle fat content and our data do support the assertion that genetic effects on lipogenic gene expression contribute to variations in lipid deposition in muscle.

The PPAR signaling pathway is known to respond to lipids and elicit transcriptional changes on genes involved in lipid metabolism in mammals. Specifically, activation of PPARγ leads to increased triglyceride accumulation in muscle and liver (Pouslen et al., 2012). It is the most abundant of the PPARs, occurring in adipose tissue at levels 30-fold higher than levels found in other tissues (Varga et al., 2011). PPARγ affects transcription rates of a variety of lipogenic target genes such as *fabp, cd36, lpl, leptin, acc, fas, and scd1* (Lee and Hossner, 2002). Additionally, PPARα and PPARβ are responsible for regulating fatty acid β-oxidation (Varga et al., 2011). Of these genes, *pparγ, srebp1, fas, scd1, fabp4, and cd36* have shown the most association with increased intramuscular fat while *pparα* shows the most association with decreased intramuscular fat in mammals as well as aquatic animals (Childs et al., 2002; Kolditz et al., 2008; Wang et al., 2009; Hocquette et al., 2010). Although PPAR transcription factors and their associated genes have potential to predict intramuscular fat, there were no effects of phenotype on any of the PPAR genes in tissues included in this study. This finding suggests that post-translational modifications of PPAR-related genes, or additional regulatory mechanisms, beyond those measured in this study, contribute to the observed phenotypes.

Although there were no specific associations of gene expression with the phenotypic groups investigated in this study, there were several notable correlations between variables measured and gene expression. GtSI was negatively correlated with *redd1* while trim losses were positively correlated with *redd1* in the visceral adipose tissue. REDD1 inhibits the TORC1 complex and thereby inhibits expression of lipogenic genes (Laplante and Sabatini 2009; Caron, 2010). However, increased GtSI appears to be more closely related to decreased β-oxidation gene expression in the visceral adipose tissue, especially with *pparβ* and *cpt1c*. These findings suggest that a reduced capacity for β-oxidation in this tissue contributes to accumulation of visceral lipid stores more so than increased lipogenesis. Fillet crude fat content is positively

correlated with lipogenic genes, *fabp3, lpl, and scd1,* in the liver. These data suggest that increased expression of lipogenic genes in the liver may account for increased muscle fat content in HF fish while increased visceral adipose tissue stores, as indicated by high GtSI, are associated with decreased β-oxidation gene expression in LF fish. With respect to indices of fillet quality, the only correlation was between *cpt1a* expression in the muscle and shear force, suggesting that increased β-oxidation gene expression in the muscle leads to a lower muscle fat content and, in turn, a firmer fillet.

In general, it appears that genes within the β-oxidation pathway of muscle and visceral adipose tissue, especially the *cpt1* isoforms, may serve as potential indicators of fillet yield and fatness. The lack of muscle yield and fat associations with genes within the fatty acid synthesis pathway may be a result of fish having negligible lipid synthesis within the muscle (Rollin et al., 2003). The absence of a correlation between fillet crude fat content and *pparγ* agrees with Childs et al. (2002) work with cattle that also did not show an association with intramuscular fat and *pparγ*. In addition, we did not observe a correlation with muscle fat and *cd36* like Kolditz et al. (2010) did in rainbow trout. Moreover, we did not observe correlations with muscle fat and *fabp4* or *me* as has been reported in pigs and cattle (Mourot and Kouba, 1999; Bonnet et al., 2007). Potential markers for increased intramuscular fat, including *lpl, fabp3,* and *scd1* expression in the liver, were associated with increased intramuscular fat; however, other potential markers including *pparγ, srebp1,* and subsequent lipogenic genes, were not associated with fillet fat content in this study as has been reported in mammals (Hausman et al., 2009). The lack of correlations with specific traits, especially with fillet fat content, could be caused by narrow differences in fat content and other traits in this study.

CONCLUSION

Data from this study provide information about the metabolism of lipid stores and its association with two important traits in fish production, fillet yield and fillet fat content. LY/LF fish produced the softest cooked fillets, and the darkest, most yellow raw fillets. Whereas HY/LF fish have the most desirable fillet characteristics including bright, firm fillets with high separable muscle yields. Additionally, HY/LF fish had the highest ω3:ω6 ratio while maintaining optimal growth, suggesting that increasing fillet yield does not negatively impact other aspects of fillet quality besides ω3:ω6 ratio. There were limited effects of phenotype on gene expression with the visceral adipose tissue having the most association with phenotype. The correlations of β-oxidation genes, specifically *cpt1* isoforms in white muscle, with fillet fat content and shear force suggests increased β-oxidation is a mechanism negatively affecting fillet fat content and fillet quality by decreasing the amount of lipid within the muscle and altering the firmness of the fillet. Overall, data suggest that differences in growth and fillet quality may result from variation in the capacity for β-oxidation. Therefore, based on results of this work it may be possible to identify specific genes, within fatty acid metabolism, as potential markers for traits of interest in the aquaculture industry.

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TABLES and FIGURES

	Phenotypic Group				P-values	
	LY/LF	**LY/HF**	**HY/LF**	**HY/HF**	**Phenotype**	**Family**
WBW (g)	772±45[a]	1000±45[b]	983±43[b]	1152±43[c]	**<0.0001**	**<0.0001**
GSI (%)	0.24±0.02[a]	0.30±0.02[ab]	0.30±0.02[b]	0.33±0.02[b]	**0.0448**	**0.0011**
HSI (%)	1.06±0.03[ab]	1.20±0.03[c]	0.98±0.03[a]	1.12±0.03[bc]	**<0.0001**	*0.0785*
GtSI (%)	7.88±0.32[ab]	9.33±0.32[c]	7.04±0.31[a]	7.95±0.31[b]	**<0.0001**	**<0.0001**
TGC	1.9±0.06[a]	2.1±0.06b[b]	2.1±0.05[b]	2.3±0.05[c]	**<0.0001**	**<0.0001**
Feed Efficiency	0.81±0.16	0.49±0.16	0.69±0.016	0.90±0.15	0.2621	0.3432
n	22	22	24	24		

TABLE 1: GROWTH RESPONSES OF PHENOTYPIC GROUPS

Values are LSmeans ± SEM. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat; WBW-whole body weight; GSI-gonadosomatic index; HIS-hepatosomatic index; GtSI-gastrointestinal tract; TGC-thermal growth coefficient.

	Phenotypic Group				P-values	
	LY/LF	**LY/HF**	**HY/LF**	**HY/HF**	**Phenotype**	**Family**
Yields						
Separable Muscle (%)	46.0±0.4^{a}	47.1±0.4^{a}	49.8±0.4^{b}	50.3±0.4^{b}	**<0.0001**	**<0.0001**
Trim Loss (%)	31.4±0.4^{b}	29.6±0.4^{a}	29.9±0.4^{a}	29.0±0.4^{a}	**<0.0001**	**<0.0001**
Fillet Thickness (mm)	19.1±0.5^{a}	21.0±0.5^{b}	20.9±0.5^{b}	22.5±0.5^{c}	**0.0002**	**<0.0001**
Cook Loss (%)	14.2±0.3^{c}	13.6±0.3^{bc}	13.2±0.3^{ab}	12.6±0.3^{a}	**0.0018**	**0.0004**
Proximate Composition						
Moisture (%)	72.4±0.3^{b}	70.1±0.3^{a}	71.8±0.3^{b}	69.7±0.3^{a}	**<0.0001**	**<0.0001**
Crude Fat (%)	7.1±0.3^{a}	9.6±0.3^{b}	6.8±0.3^{a}	9.9±0.3^{b}	**<0.0001**	**<0.0001**
Crude Protein (%)	20.4±0.1	20.4±0.1	20.6±0.1	20.7±0.1	0.4949	0.6119
Ash (%)	1.4±0.02^{a}	1.5±0.02^{b}	1.4±0.02^{a}	1.5±0.02^{b}	**0.0058**	**0.0155**
Texture						
Peak Force (g force/g sample)	413±17^{a}	475±17^{b}	454±16^{b}	455±16^{b}	*0.0793*	0.1237
Energy (kg/mm)	130.8±8.6^{a}	170.3±8.6^{b}	166.5±8.3^{b}	182.2±8.3^{b}	**0.0004**	**<0.0001**
Color						
L*	42.8±0.4^{a}	43.9±0.4^{b}	42.4±0.4^{a}	43.6±0.4^{b}	**0.0448**	*0.0798*
a	1.1±0.1	1.1±0.1	1.3±0.1	1.3±0.1	0.3684	0.1096
b	3.4±0.2^{a}	4.0±0.2^{b}	3.3±0.2^{a}	4.1±0.2^{b}	**0.0091**	**0.0388**
n	22	22	24	24		

TABLE 2: FILLET CHARACTERISTICS OF PHENOTYPIC GROUPS

Values are LSmeans ± SEM. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat.

Fatty Acid mg / g tissue	Phenotypic Group				P-values	
	LY/LF	LY/HF	HY/LF	HY/HF	Phenotype	Family
12:0	0.012±0.002[a]	0.012±0.002[a]	0.010±0.002[a]	0.019±0.002[b]	**0.0034**	0.2372
14:0	0.58±0.07	0.65±0.07	0.57±0.07	0.81±0.07	*0.0651*	0.4276
14:1	0.016±0.003[a]	0.019±0.003[ab]	0.016±0.002[a]	0.025±0.002[b]	**0.0484**	0.2158
15:0	0.049±0.005	0.050±0.005	0.050±0.005	0.062±0.005	0.2472	0.4291
16:0	3.94±0.48	4.58±0.48	3.90±0.46	5.47±0.46	*0.0663*	0.4508
16:1	1.38±0.17[a]	1.69±0.17[ab]	1.32±0.17[a]	2.00±0.17[b]	**0.0178**	0.2991
17:0	0.042±0.005	0.044±0.005	0.044±0.005	0.055±0.005	0.2523	0.4921
18:0	0.85±0.11	0.97±0.11	0.83±0.10	1.18±0.10	*0.0609*	0.4548
18:1n-9	4.71±0.59[a]	5.60±0.59[ab]	4.60±0.56[a]	6.63±0.56[b]	**0.0479**	0.5080
18:2n-6	2.94±0.32	2.89±0.32	2.84±0.31	3.62±0.31	0.2427	0.4689
18:3n-6	0.048±0.006	0.053±0.006	0.045±0.006	0.058±0.006	0.4027	0.7088
18:3n-3	0.29±0.03	0.28±0.03	0.28±0.03	0.37±0.03	0.1346	0.2653
20:0	0.20±0.003[a]	0.024±0.003[ab]	0.021±0.002[a]	0.029±0.002[b]	**0.0350**	0.2195
20:1	0.43±0.05	0.47±0.05	0.42±0.5	0.58±0.05	*0.0964*	0.2984
20:2	0.23±0.03	0.24±0.03	0.22±0.03	0.27±0.03	0.7358	0.5758
20:3n-6	0.14±0.02	0.15±0.02	0.13±0.02	0.16±0.02	0.6573	0.2583
20:3n-3	0.033±0.004	0.028±0.004	0.029±0.004	0.037±0.004	0.3002	0.3318
20:4n-6	0.18±0.02	0.19±0.02	0.19±0.02	0.23±0.02	0.3378	0.5457
20:5n-3	0.54±0.06	0.57±0.06	0.56±0.06	0.68±0.06	0.2670	0.4263
22:1n-9	0.048±0.006	0.051±0.006	0.047±0.006	0.061±0.006	0.4041	0.3941
22:6n-3	1.88±0.19	1.94±0.19	1.97±0.18	2.15±0.18	0.7726	0.4064
24:1	0.050±0.005	0.048±0.005	0.044±0.005	0.059±0.005	0.1653	0.3599
SFA	5.50±0.67	6.33±0.67	5.43±0.64	7.63±0.64	*0.0655*	0.4509
MUFA	6.63±0.82[a]	7.88±0.82[ab]	6.44±0.79[a]	9.35±0.79[b]	**0.0410**	0.4542
PUFA	6.28±0.66	6.34±0.66	6.27±0.63	7.57±0.63	0.3955	0.4527
ω3	2.74±0.28	2.81±0.28	2.84±0.27	3.23±0.27	0.5787	0.4144
ω6	3.54±0.39	3.53±0.39	3.43±0.37	4.34±0.37	0.2853	0.4910
ω3: ω6	0.82±0.03[b]	0.80±0.03[ab]	0.85±0.03[b]	0.74±0.03[a]	**0.0301**	0.0877
n	22	22	24	24		

TABLE 3: FILLET FATTY ACID COMPOSITION OF PHENOTYPIC GROUPS

Values are LSmeans ± SEM. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat.

Gene	Liver Phenotype	Liver Family	Liver n (89)	White Muscle Phenotype	White Muscle Family	White Muscle n (84)	Visceral Adipose Tissue Phenotype	Visceral Adipose Tissue Family	Visceral Adipose Tissue n (82)
Fatty Acid Synthesis									
gpat	0.7474	0.4993	84	0.8439	0.9150	84	**0.0031**	0.5562	79
srebp1	0.2046	0.8491	53	0.7362	0.6046	15	ND	ND	0
acyl	0.1001	0.2663	72	0.6313	0.8049	66	0.7623	0.1755	52
acc	0.6085	0.8342	82	0.4191	0.8500	81	0.7539	0.6657	72
fas	0.4338	0.9372	87	0.2370	0.5599	84	0.9158	0.1119	78
scd1	**0.0239**	0.5736	79	0.8066	0.8311	52	0.0994	0.3318	39
β-Oxidation									
magl	0.2564	0.2665	82	0.0656	0.2173	84	0.4953	**0.0377**	79
cpt1a	0.4710	0.2734	89	0.2246	0.3653	84	0.2089	0.1485	59
cpt1b	0.7945	0.4475	50	0.8391	0.4955	84	0.3954	0.1388	74
cpt1c	0.5700	0.9939	89	0.2814	0.7187	84	0.3649	0.2036	82
cpt1d	0.8200	0.6973	30	0.0878	**0.0372**	72	0.7639	0.5674	82
cpt2	0.9183	0.9557	84	0.7585	0.3786	83	0.8383	0.4608	14
aco	0.2189	0.7182	86	0.3186	0.4389	84	0.9252	0.6317	81
acdh	0.1655	0.5036	83	0.2372	0.2436	80	0.9351	0.2814	67
ehhadh	0.2367	0.7476	87	0.2491	**0.0003**	84	0.4287	0.1822	82
acat2	0.6491	0.5901	71	0.6688	0.3031	84	0.1690	0.5286	81
Fatty Acid Transport									
fabp3	0.3040	0.8865	87	0.7340	0.7480	75	0.1994	0.5384	79
fabp4	0.1516	0.6020	76	0.4375	0.4474	74	0.7385	0.5401	75
lpl	0.4155	0.4099	88	0.6189	0.8641	67	0.9224	0.5166	82
cd36	0.9803	0.4588	89	0.2726	0.0898	84	0.5102	0.5011	81
me	0.4732	0.3539	89	0.4994	0.3266	84	0.8171	0.6178	56
Signaling Factors									
erk	0.1554	0.7829	70	0.2034	0.3513	82	0.6256	0.0857	81
akt2	ND	ND	0	0.9395	0.9454	16	**0.0336**	0.2125	75
redd1	0.1625	**0.0233**	89	0.8592	0.7764	84	0.4187	0.4868	82
mo25	0.1020	0.3337	89	0.2192	**0.0017**	84	0.3210	0.9915	81
mtor	0.9277	0.9107	87	0.3271	0.5877	84	0.7633	0.5098	82
raptor	0.9657	0.8474	89	0.9279	0.6232	16	0.3983	0.2057	15
rictor	0.9301	0.3865	88	0.5246	0.2748	82	**0.0018**	0.3968	80
pras40	0.9974	0.8620	89	0.3899	0.6001	83	0.4677	0.6814	82
pparβ	0.1493	0.8875	84	0.5968	0.6208	84	0.7212	0.2938	82
pparγ	0.7598	0.5726	89	0.2478	0.6274	23	0.2891	0.0744	55
rxr	0.1523	0.4325	79	0.4481	0.4998	83	0.1380	0.1043	81

TABLE 4: SIGNIFICANCE AND N-VALUES FOR ALL GENES TARGETED BY THE MULTIPLEX IN LIVER, WHITE MUSCLE, AND VISCERAL ADIPOSE TISSUE OF PHENOTYPIC GROUPS

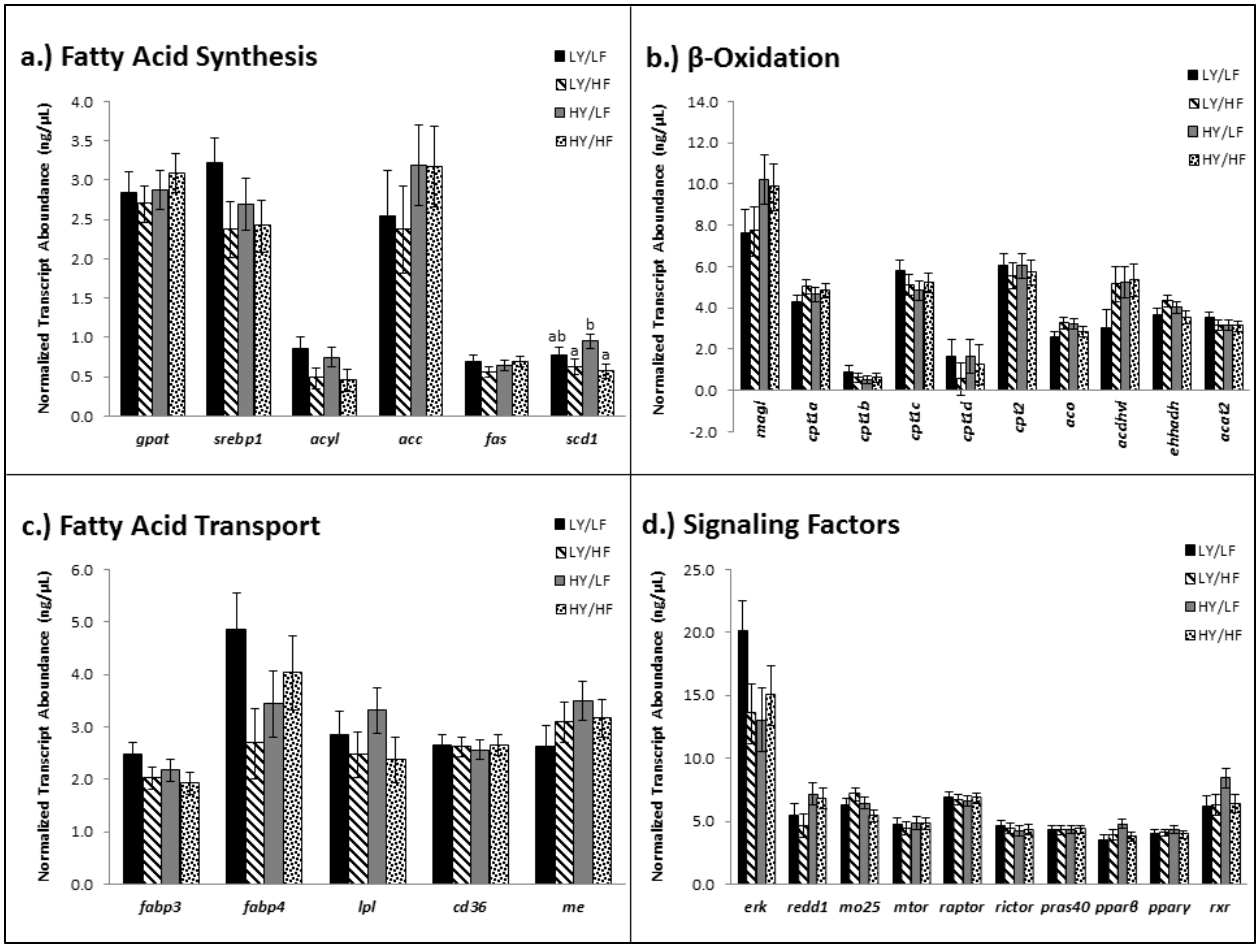

FIGURE 1: LIVER GENE EXPRESSION OF PHENOTYPIC GROUPS

a) Phenotype effects on genes within fatty acid synthesis expressed in the liver; b) Phenotype effects on genes within β-oxidation expressed in the liver; c) Phenotype effects on genes within fatty acid transport expressed in the liver; d.) Phenotype effects on signaling factor genes expressed in the muscle. Values are LSmeans ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat.

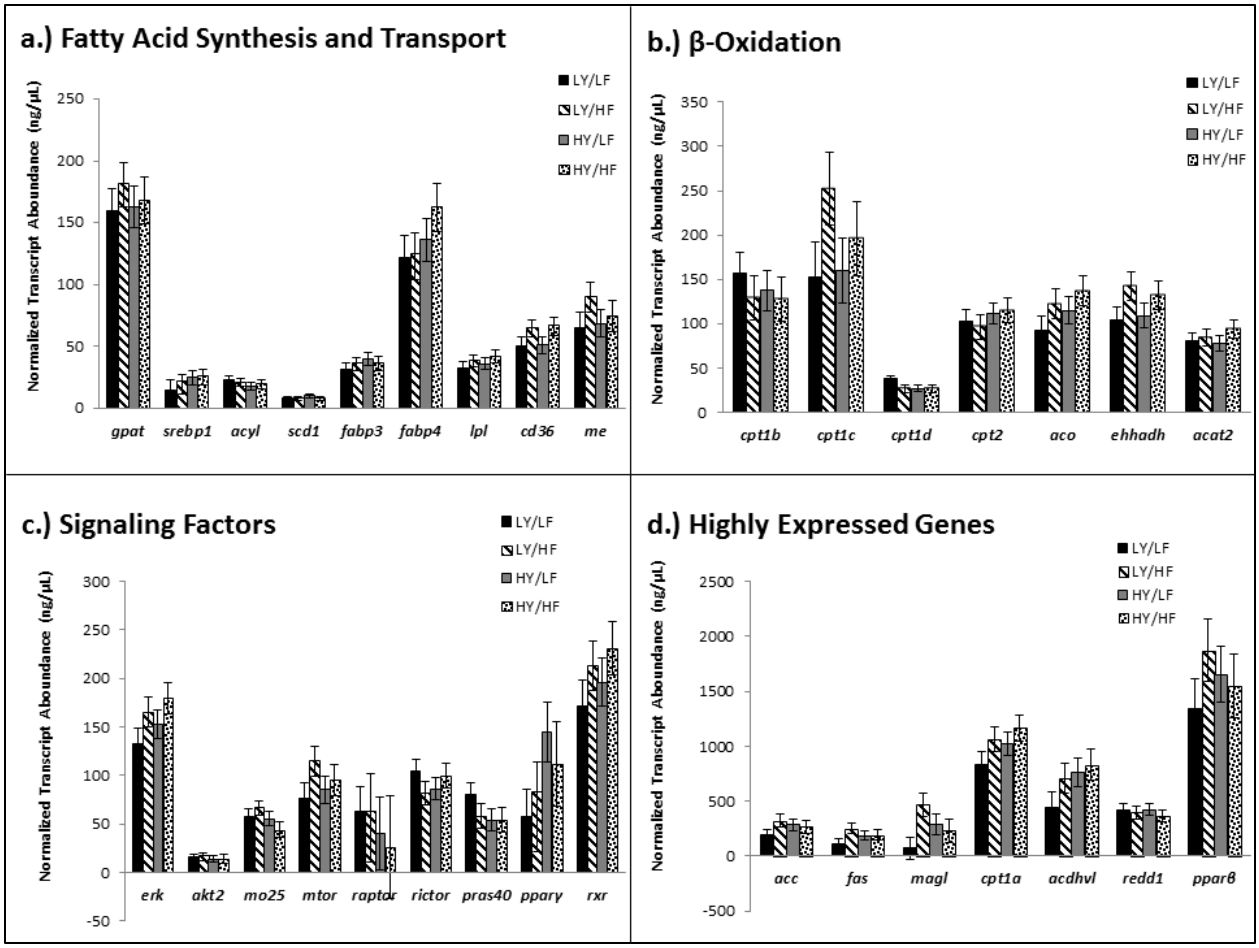

FIGURE 2: WHITE MUSCLE GENE EXPRESSION OF PHENOTYPIC GROUPS

a) Phenotype effects on genes within fatty acid synthesis and transport expressed in the muscle; b) Phenotype effects on genes within β-oxidation expressed in the muscle; c) Phenotype effects on signaling factor genes expressed in the muscle; d.) Phenotype effects on genes with high levels of expression in muscle. Values are LSmeans ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat.

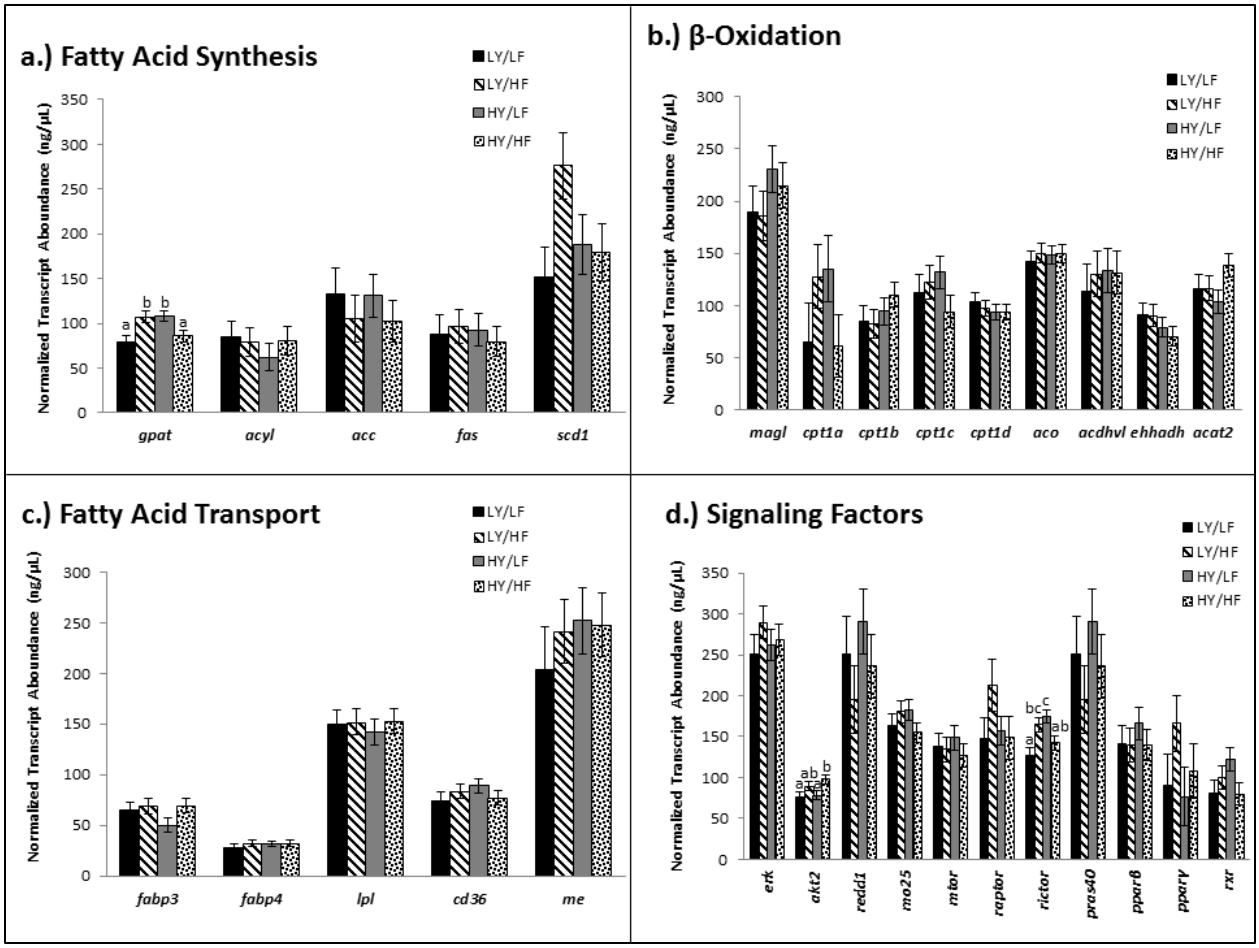

FIGURE 3: VISCERAL ADIPOSE TISSUE GENE EXPRESSION OF PHENOTYPIC GROUPS

a) Phenotype effects on genes within fatty acid synthesis expressed in the visceral adipose tissue; b) Phenotype effects on genes within β-oxidation expressed in the visceral adipose tissue; c) Phenotype effects on genes within fatty acid transport in the visceral adipose tissue; d.) Phenotype effects on signaling factor genes expressed in the visceral adipose tissue. Values are LSmeans ± SEM and represent the fold change in gene abundance, relative to the normalized mean of three reference genes (*β-actin, eF1a, and rplp2)*. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat.

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DISSERTATION CONCLUSION

Maturation is a dominant physiological process that causes a restructuring of metabolism. In 2N and 3N females, phenotypic differences occur at 21 M while differences in gene expression occur at 20 M. Diploid and triploid females also have differing fatty acid metabolisms during sexual maturation. Diploids are mobilizing lipid stores to support gonadogenesis while 3Ns are synthesizing fatty acids to store excess energy. There is increased expression of β-oxidation genes in 2N female muscle and visceral adipose tissue by 20 M, and these findings are consistent with increased *pparβ* expression. On the other hand, 3N livers have increased expression of fatty acid synthesis genes at 20 M consistent with increased *pparγ* expression. Therefore, maturing diploid females appear to increase lipid utilization at 20 M and should be harvested prior to this point. In addition, immature male and female rainbow trout appear to have differing fatty acid metabolisms prior to maturation. Females have increased fillet PUFA content along with increased hepatic expression of fatty acid synthesis genes. Males have increased expression of β-oxidation genes within muscle. Differences in gene expression were also associated with altered expression within the mTOR signaling pathway. These variations in gene expression may contribute to the compositional differences observed between genders and further supports the concept of culturing all-female trout for food production. Moreover, there is evidence that variation in β-oxidation gene expression could be responsible for differences observed in fillet yield and composition. It may be possible to identify genes within fatty acid metabolism as potential markers for fillet quality traits in a study with greater differences in fat content. Overall, fatty acid metabolism is significantly altered by sexual maturation, polyploidy, and gender in rainbow trout. These factors should therefore be considered when culturing fish in order to optimize growth, fillet quality, and profitability.

APPENDICIES

APPENDIX 1

FEEDING RATE STUDY TISSUE FATTY ACID COMPOSITIONS (mg/g sample)

Table 1: Feeding Rate Study Muscle Fatty Acid Composition (mg/g sample)

	Ration Effects					Ploidy Effects	
mg/g	0.25% Ration	0.5% Ration	Satiation	Ration P-value	Family P-value	3N	Ploidy P-value
12:0	0.002±0.001	0.002±0.001	0.003±0.001	0.5875	0.0261	0.006±0.001	0.0098
14:0	0.28±0.03	0.26±0.04	0.44±0.04	0.0039	0.0458	0.50±0.05	0.0155
14:1	0.005±0.001	0.003±0.001	0.007±0.001	0.0260	0.1360	0.010±0.001	0.0033
15:0	0.022±0.002	0.020±0.003	0.035±0.003	0.0011	0.0991	0.03±0.003	0.0258
16:0	1.68±0.17	1.45±0.21	2.48±0.18	0.0020	0.0845	2.69±0.28	0.0243
16:1	0.43±0.05	0.38±0.06	0.68±0.05	0.0030	0.0158	0.81±0.08	0.0120
17:0	0.021±0.002	0.019±0.003	0.033±0.002	0.0013	0.2834	0.029±0.003	0.0659
18:0	0.30±0.03	0.26±0.04	0.46±0.03	0.0015	0.1438	0.48±0.05	0.0310
18:1n-9	1.37±0.15	1.24±0.19	2.12±0.16	0.0031	0.0353	2.05±0.26	0.1270
18:2n-6	1.16±0.12	1.03±0.16	1.82±0.13	0.0013	0.0573	1.57±0.16	0.0807
20:0	0.011±0.001	0.010±0.002	0.018±0.001	0.0046	0.0869	0.018±0.002	0.0381
18:3n-6	0.024±0.004	0.025±0.005	0.040±0.005	0.0437	0.0818	0.039±0.004	0.1543
20:1	0.43±0.05	0.33±0.06	0.61±0.05	0.0036	0.0171	0.59±0.03	0.0146
18:3n-3	0.12±0.01	0.11±0.02	0.21±0.01	0.0003	0.0371	0.18±0.02	0.0501
20:2	0.12±0.02	0.14±0.02	0.22±0.02	0.0026	0.0472	0.17±0.02	0.6003
22:0	0.007±0.003	0.012±0.004	0.014±0.003	0.3980	0.5040	0.011±0.004	0.7299
20:3n-6	0.10±0.01	0.09±0.02	0.15±0.01	0.0124	0.0037	0.12±0.02	0.3773
22:1n-9	0.031±0.004	0.028±0.005	0.043±0.004	0.0655	0.0007	0.050±0.005	0.0074
20:3n-3	0.019±0.003	0.016±0.004	0.023±0.003	0.4076	0.3182	0.019±0.002	0.5685
20:4n-6	0.14±0.01	0.09±0.02	0.16±0.01	0.0110	0.0786	0.16±0.02	0.0566
20:5n-3	0.38±0.03	0.24±0.04	0.45±0.03	0.0019	0.5054	0.37±0.04	0.0654
24:1	0.047±0.005	0.035±0.006	0.059±0.005	0.0190	0.4753	0.055±0.007	0.1146
22:6n-3	1.72±0.15	1.24±0.18	2.04±0.16	0.0122	0.4963	1.81±0.20	0.1282
SAT	2.31±0.24	2.04±0.29	3.48±0.25	0.0021	0.0831	3.76±0.39	0.0244
MUFA	2.32±0.25	2.02±0.31	3.52±0.27	0.0030	0.0237	3.56±0.39	0.0446
PUFA	3.79±0.34	2.99±0.42	5.12±0.36	0.0028	0.1606	4.44±0.47	0.1031
ω3	2.24±0.19	1.61±0.23	2.72±0.20	0.0066	0.4204	2.38±0.26	0.1075
ω6	1.54±0.16	1.38±0.20	2.40±0.17	0.0013	0.0563	2.06±0.22	0.1083
ω3: ω6	1.47±0.06	1.19±1.18	1.18±0.06	0.0046	0.0911	1.17±0.05	0.9606
n	12	10	11			12	

Table 2: Feeding Rate Study Liver Fatty Acid Composition (mg/g sample)

	Ration Effects					Ploidy Effects	
mg/g	0.25% Ration	0.5% Ration	Satiation	Ration P-value	Family P-value	3N	Ploidy P-value
12:0	ND	ND	ND	ND	ND	ND	ND
14:0	0.26±0.02[b]	0.24±0.02[ab]	0.18±0.02[a]	0.0321	0.4064	0.20±0.02	0.0515
14:1	ND	ND	ND	ND	ND	ND	ND
15:0	0.04±0.01	0.03±0.01	0.03±0.01	0.1113	0.2650	0.01±0.001*	<0.0001
16:0	2.64±0.16	2.57±0.19	2.47±0.17	0.7498	0.0933	2.57±0.17	0.9554
16:1	0.32±0.03	0.26±0.04	0.29±0.04	0.5589	0.5217	0.57±0.06*	0.0158
17:0	0.06±0.01	0.05±0.01	0.05±0.01	0.0708	0.0449	0.02±0.002*	<0.0001
18:0	1.36±0.09	1.36±0.11	1.10±0.09	0.1191	0.0927	0.87±0.07*	0.0009
18:1n-9	1.49±0.13	1.36±0.16	1.45±0.14	0.8323	0.2349	2.18±0.20*	0.0337
18:2n-6	0.88±0.07	0.78±0.09	0.78±0.07	0.5361	0.1906	0.77±0.06	0.9473
20:0	0.01±0.002	0.01±0.003	0.01±0.002	0.9674	0.4345	0.03±0.003*	0.0021
18:3n-6	0.03±0.003[b]	0.02±0.004[ab]	0.01±0.004[a]	0.0365	0.2380	0.03±0.004	0.0803
20:1	0.51±0.04	0.59±0.06	0.42±0.05	0.0859	0.0225	0.50±0.04	0.1474
18:3n-3	0.06±0.01	0.04±0.01	0.06±0.01	0.4159	0.3327	0.04±0.01	0.8635
20:2	0.38±0.04	0.50±0.04	0.40±0.04	0.0922	<0.0001	0.32±0.04*	0.0381
22:0	ND	ND	ND	ND	ND	ND	ND
20:3n-6	0.45±0.06	0.51±0.07	0.34±0.06	0.1734	0.3161	0.36±0.04*	0.0405
22:1n-9	ND	ND	ND	ND	ND	ND	ND
20:3n-3	0.03±0.003	0.03±0.004	0.03±0.004	0.9943	0.2234	0.04±0.01	0.0882
20:4n-6	0.75±0.13	0.59±0.16	0.62±0.13	0.6853	0.0545	0.40±0.05*	0.0077
20:5n-3	0.69±0.09	0.43±0.11	0.58±0.09	0.1924	0.1309	0.40±0.05	0.4513
24:1	0.18±0.01	0.19±0.02	0.21±0.01	0.2915	0.2452	0.25±0.02	0.1013
22:6n-3	3.32±0.37	2.86±0.46	2.92±0.39	0.6751	0.0545	2.73±0.27	0.4831
SFA	4.38±0.27	4.27±0.33	3.84±0.28	0.3787	0.1292	3.69±0.26	0.2187
MUFA	2.51±0.21	2.41±0.26	2.37±0.22	0.8943	0.2115	3.50±0.29	0.0572
PUFA	6.59±0.67	5.78±0.83	5.74±0.71	0.6310	0.0484	5.10±0.46	0.2296
ω3	4.10±0.46	3.37±0.57	3.58±0.49	0.5735	0.0630	3.21±0.32	0.4934
ω6	2.49±0.24	2.40±0.30	2.16±0.25	0.6403	0.0516	1.89±0.16	0.0528
ω3: ω6	1.71±0.13	1.39±0.16	1.63±0.14	0.3153	0.7513	1.66±0.10	0.2054
n	12	10	11			12	

Table 3: Feeding Rate Study Visceral Adipose Tissue Fatty Acid Composition (mg/g sample)

	Ration Effects					Ploidy Effects	
mg/g	0.25% Ration	0.5% Ration	Satiation	Ration P-value	Family P-value	3N	Ploidy P-value
12:0	0.19±0.02	0.20±0.02	0.21±0.02	0.8218	0.9194	0.27±0.02*	0.0239
14:0	14.64±1.07	13.23±1.33	14.53±1.14	0.6804	0.7981	17.14±1.23	0.0727
14:1	0.42±0.03	0.39±0.04	0.43±0.04	0.7611	0.7888	0.59±0.04*	0.0068
15:0	1.71±0.13	1.59±0.16	1.81±0.13	0.5797	0.5428	1.59±0.15*	0.0439
16:0	25.18±1.90	23.74±2.36	26.97±2.02	0.5905	0.6279	38.31±2.15*	0.0016
16:1	40.42±3.16	37.69±3.92	42.64±3.35	0.6410	0.5020	52.54±3.50*	0.0338
17:0	1.37±0.10	1.20±0.12	1.36±0.10	0.5067	0.5012	1.67±0.12*	0.0395
18:0	8.66±0.50	6.92±0.62	8.06±0.53	0.1215	0.3347	11.12±0.58*	0.0012
18:1n-9	67.14±4.40	57.05±5.46	64.32±4.67	0.3686	0.2480	72.27±5.02	0.1204
18:2n-6	112.66±8.41	102.97±10.44	113.61±8.93	0.7090	0.6629	108.61±9.84	0.7418
20:0	0.48±3.71	0.98±4.61	0.21±3.98	0.2279	0.2661	0.46±4.67	0.3441
18:3n-6	1.88±1.18	1.57±0.22	1.84±0.19	0.5207	0.2596	2.36±0.16*	0.0278
20:1	45.02±4.08[c]	19.6±5.06[a]	32.30±4.33[b]	0.0031	0.7065	33.22±5.10	0.1160
18:3n-3	12.22±1.32	11.48±1.64	14.15±1.41	0.4357	0.7307	15.50±1.46	0.1306
20:2	7.20±0.67	6.46±0.83	6.83±0.71	0.6583	0.1808	6.87±0.79	0.6870
22:0	ND	ND	ND	ND	ND	ND	ND
20:3n-6	4.46±0.35	4.07±0.43	3.83±0.37	0.4750	0.0003	3.63±0.42	0.5874
22:1n-9	ND	ND	ND	ND	ND	ND	ND
20:3n-3	ND	ND	ND	ND	ND	ND	ND
20:4n-6	2.94±0.31	3.06±0.38	2.78±0.33	0.8527	0.5977	3.47±0.36	0.4485
20:5n-3	6.60±0.66	5.93±0.82	7.30±0.70	0.4640	0.6019	9.41±0.65*	0.0134
24:1	1.41±0.41	1.73±0.51	1.06±0.44	0.6143	0.2164	1.23±0.53	0.7262
22:6n-3	18.90±1.81	14.85±2.24	19.30±1.92	0.2878	0.8772	23.99±1.92*	0.0265
SFA	52.24±5.39	56.69±6.69	52.91±5.72	0.8656	0.2245	71.09±6.67	0.1751
MUFA	154.41±10.05	116.46±12.47	140.75±10.67	0.0841	0.3890	159.86±11.54*	0.0484
PUFA	166.85±12.15	150.38±5.07	169.19±12.89	0.6093	0.6793	173.85±13.73	0.3556
ω3	37.71±3.61	32.25±4.49	40.75±3.84	0.3764	0.9804	48.90±3.86*	0.0364
ω6	129.14±9.48	118.13±11.76	128.44±10.06	0.7401	0.5528	124.95±11.18	0.7206
ω3: ω6	0.29±0.02	0.30±0.02	0.32±0.02	0.5434	0.6410	0.39±0.02*	0.0227
n	12	10	11			12	

Table 4: Feeding Rate Study Diploid Gonad Fatty Acid Composition (mg/g sample)

	Ration Effects				
mg/g	0.25% Ration	0.5% Ration	Satiation	Ration P-value	Family P-value
12:0	0.02±0.001	0.02±0.002	0.02±0.001	0.0574	<0.0001
14:0	1.70±0.07	1.83±0.09	1.70±0.08	0.4532	0.0034
14:1	0.03±0.002	0.03±0.002	0.03±0.002	0.1054	0.0051
15:0	0.19±0.01	0.20±0.01	0.18±0.01	0.2945	0.0425
16:0	10.87±0.33	11.14±0.43	10.25±0.37	0.2768	0.0801
16:1	2.99±0.13	3.04±0.17	2.97±0.14	0.9483	0.0034
17:0	0.17±0.005[b]	0.16±0.007[b]	0.15±0.006[a]	0.0178	0.2339
18:0	2.99±0.09	3.29±0.12	3.16±0.10	0.1241	0.0527
18:1n-9	9.53±0.31	10.14±0.42	9.19±0.36	0.5318	0.0003
18:2n-6	6.25±0.24	6.49±0.32	5.98±0.27	0.4896	0.0008
20:0	0.03±0.001	0.03±0.001	0.02±0.001	0.1430	0.0610
18:3n-6	0.25±0.02[b]	0.17±0.02[a]	0.14±0.02[a]	0.0002	0.0974
20:1	1.53±0.05	1.74±0.07	1.63±0.06	0.0705	0.0004
18:3n-3	0.78±0.03	0.81±0.05	0.79±0.04	0.8817	0.0032
20:2	0.97±0.05[a]	1.32±0.07[b]	1.22±0.06[b]	0.0008	0.0001
22:0	0.02±0.004	0.03±0.005	0.02±0.004	0.1997	0.0051
20:3n-6	1.19±0.09	1.30±0.13	1.22±0.11	0.7782	0.0089
22:1n-9	0.07±0.003	0.08±0.004	0.07±0.004	0.0799	0.0011
20:3n-3	0.11±0.005	0.12±0.007	0.11±0.006	0.2151	0.1606
20:4n-6	1.48±0.06	1.33±0.08	1.32±0.06	0.1275	<0.0001
20:5n-3	2.25±0.07[b]	1.94±0.09[b]	1.87±0.08[a]	0.0021	0.4930
24:1	0.17±0.007	0.19±0.009	0.18±0.008	0.3854	0.0055
22:6n-3	9.60±0.30	10.01±0.40	9.75±0.34	0.7290	0.6707
SFA	15.96±0.45	16.68±0.60	15.48±0.51	0.3384	0.0371
MUFA	14.45±0.48	15.41±0.64	14.18±0.55	0.3356	0.0003
PUFA	22.91±0.65	23.51±0.88	22.40±0.73	0.6260	0.0260
ω3	12.74±0.38	12.87±0.49	12.51±0.43	0.8552	0.4662
ω6	10.16±0.33	10.64±0.44	9.89±0.38	0.4443	0.0010
ω3: ω6	1.26±0.03	1.23±0.04	1.28±0.04	0.7108	0.0013
n	12	10	11		

APPENDIX 2

PATHWAYS WITH MULITPLEX-PCR TARGET GENES HIGHLIGHED

Figure 1: mTOR Signaling Pathway with Multiplex PCR Target Genes Highlighted

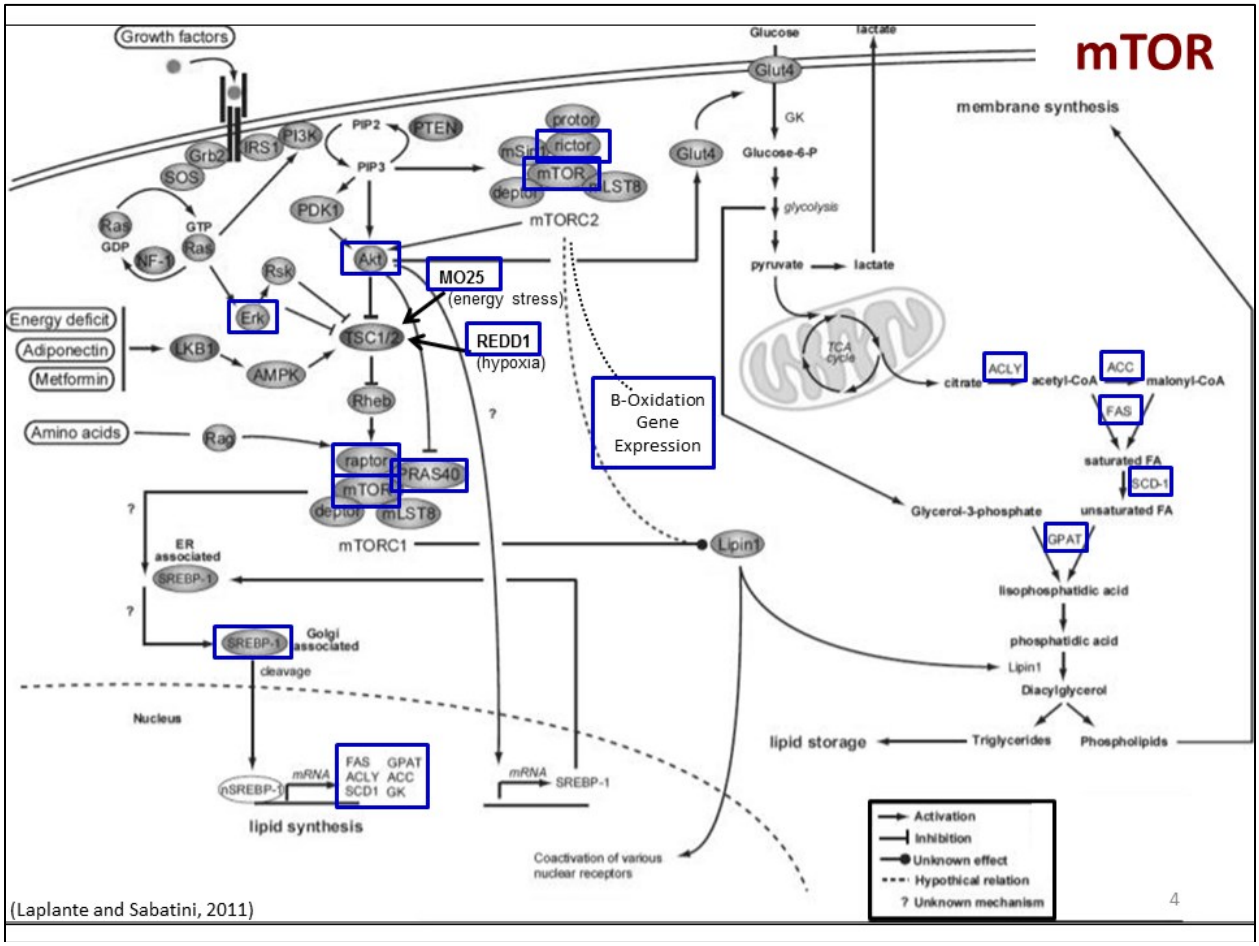
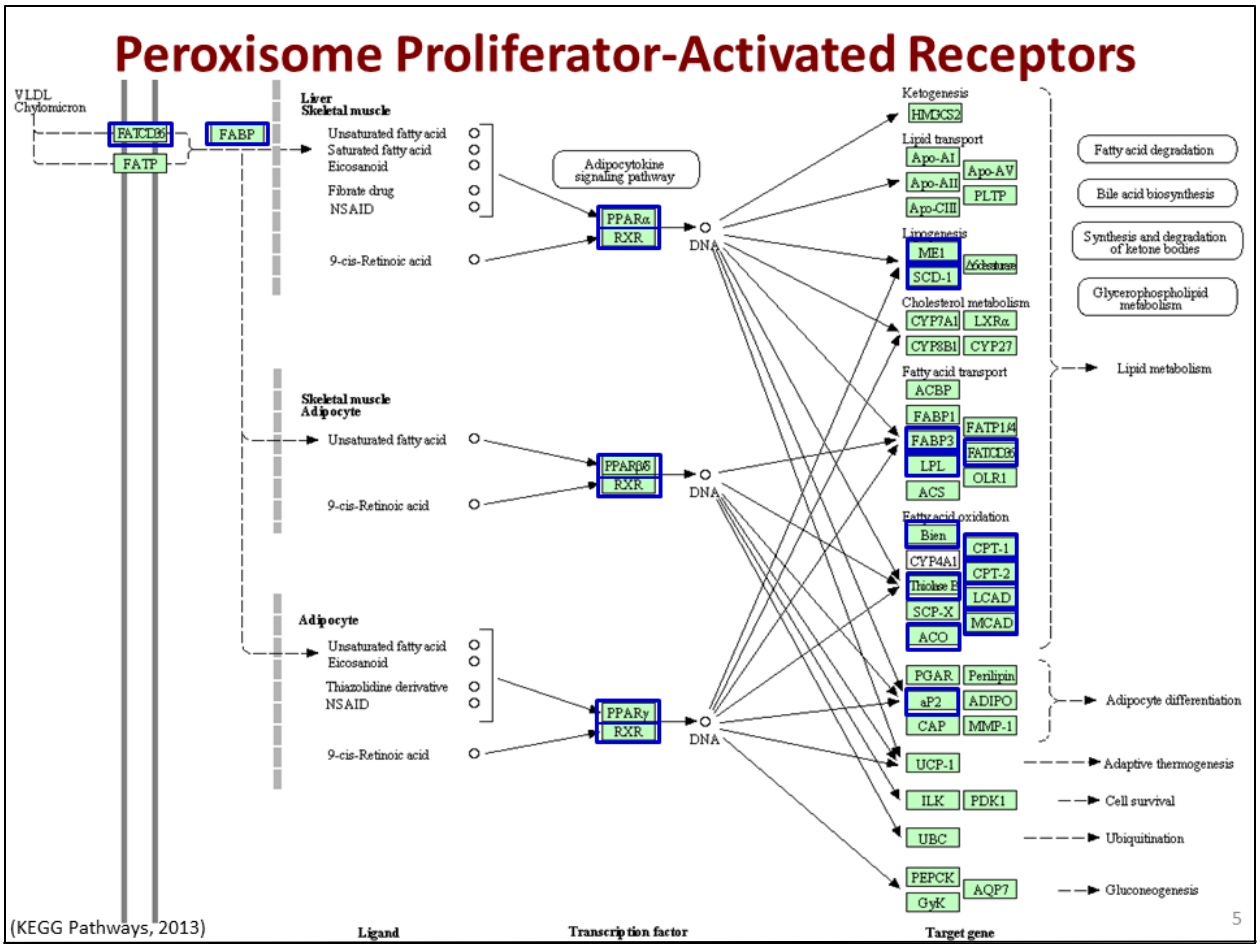


Figure 2: PPAR Signaling Pathway with Multiplex PCR Target Genes Highlighted



APPENDIX 3

TABLE 5: Primer Sequences and Expected PCR Products for Multiplex-PCR Reactions

Gene	Primer Sequences (5'-3')	PCR Product Length (bp)
REFERENCE GENES		
βactin	AGGTGACACTATAGAATAGATCCGGTATGTGCAAAGC	217
	GTACGACTCACTATAGGGAGCTCGATGGGGTACTTCAGA	
Ef1a	AGGTGACACTATAGAATATTAAGCAACCATGGGAAAGG	301
	GTACGACTCACTATAGGGATACCTGCCGGTCTCAAACTT	
gapdh	AGGTGACACTATAGAATAGGAATCAAAGTCGTTGCCAT	287
	GTACGACTCACTATAGGGAAAGAGGCCTTGTCAATGCTG	
rplp2	AGGTGACACTATAGAATAACCGACGTTTCGTGTCTGTA	192
	GTACGACTCACTATAGGGACCTTGTCTAGGCGCTCATCT	
TARGET GENES		
acat2	AGGTGACACTATAGAATAGGATTCAGAGGTGGTGCTGT	272
	GTACGACTCACTATAGGGACTGGGTCTGGTGAGCGTATT	
acc	AGGTGACACTATAGAATAGGACAAAGAAGCGGTAGTCG	152
	GTACGACTCACTATAGGGAATGCGTAACTTTGCCCTGAC	
acdh	AGGTGACACTATAGAATATGTTCCAGTCGTGTGTACCAG	379
	GTACGACTCACTATAGGGAAGTGCTCAGGGTCACGAGAG	
acdhm	AGGTGACACTATAGAATATCTGAACTCCTGTGCAACCA	369
	GTACGACTCACTATAGGGACGTCCGTGTCGTAAAATCCT	
acdhvl	AGGTGACACTATAGAATACTAGAGCCCACAAGGACTGC	167
	GTACGACTCACTATAGGGAAGGCTACATGTGCACCATCA	
aco	AGGTGACACTATAGAATAAGGCATCGAGAAGACCAAAA	177
	GTACGACTCACTATAGGGAGGATCATCTGGGCACTCTTC	
acyl	AGGTGACACTATAGAATACCCCATAAAGGAGCATGAGA	262
	GTACGACTCACTATAGGGAGAGTTGAGCAGGAAGTTGGC	
akt2	AGGTGACACTATAGAATAGCAGAAAACGGTCGAAACTC	329
	GTACGACTCACTATAGGGAGAGACAAGCTCTGGACGGAC	
apt1a	AGGTGACACTATAGAATAGCACTGCAAAGGAGACATCA	188
	GTACGACTCACTATAGGGAATCAAACAGACAGCCATGAA	
apt1b	AGGTGACACTATAGAATAACACAGAACACAGAGGTTTAGCC	312
	GTACGACTCACTATAGGGACTTAAATCTTATGGCGCGCTTT	
apt1c	AGGTGACACTATAGAATAAATGTGCTCAGCGCAATATG	172
	GTACGACTCACTATAGGGAATGGGCACCTTGAAGTAACG	
cpt1d	AGGTGACACTATAGAATATTGACATGAAGAACCACCCA	252
	GTACGACTCACTATAGGGAAGCTGGGGTAAGCACAGAAA	
cpt2	AGGTGACACTATAGAATAGATCCTGGCTGATAAGAGCG	207
	GTACGACTCACTATAGGGACGTCGAGGGTTACGAAGAAG	
ehhadh	AGGTGACACTATAGAATAAGCTGCTGGAGGTGGTGTAT	181
	GTACGACTCACTATAGGGAGTATGGCTTCAACATCCGGT	
erk	AGGTGACACTATAGAATAAACCCAAGATCCCTTGGAAC	268
	GTACGACTCACTATAGGGATGAGCTCCTTCAGCTTCTCC	
fabp3	AGGTGACACTATAGAATACACATGGAACCTGAAGGACA	403
	GTACGACTCACTATAGGGATAGGAGCGTGTGGAGACGAC	
fabp4	AGGTGACACTATAGAATAGGGGACAAAGTGGTCGTAAA	281
	GTACGACTCACTATAGGGATACGCACTGCCACAATGTCT	
fas	AGGTGACACTATAGAATAGCTATCCTGGCAGCCTACTG	142
	GTACGACTCACTATAGGGACTGAGCCTTACACTCTGCCC	
cd36	AGGTGACACTATAGAATACTGTCACCCAGATTGGACCT	212
	GTACGACTCACTATAGGGAGCTGGGATGTTGACTGTCCT	
gpat	AGGTGACACTATAGAATAGGTCTCCCGCTTTATCTTCC	230
	GTACGACTCACTATAGGGACCAAAAAGGTCCTCCTGTGA	
lpl	AGGTGACACTATAGAATACTTGTGAAGTGCTCCCATGA	341
	GTACGACTCACTATAGGGATCATGGGCTGCTCAGTGTAG	
magl	AGGTGACACTATAGAATATCAGTTCATCCCCTTTCCTG	202
	GTACGACTCACTATAGGGAGGCCTAAGGTCAAGACACGA	
me	AGGTGACACTATAGAATACAAAAGACGAGGCTCTGAGG	197
	GTACGACTCACTATAGGGAATGGCTGTGGGTTTGAGTTC	
mo25	AGGTGACACTATAGAATATGATGAACCTTCTTCGGGAC	222
	GTACGACTCACTATAGGGAATTGAACTGTTCGTCCTCCG	
mtor	AGGTGACACTATAGAATATCTCATTGGCTCAGCAGTGT	157
	GTACGACTCACTATAGGGAACAGCATCTGACACGACACC	
pparα	AGGTGACACTATAGAATACAGGGAGGTGGAGGACCCCC	410
	GTACGACTCACTATAGGGAATACACGCCGTACTTCAGCAGA	
pparβ	AGGTGACACTATAGAATAAGCTGGATGACAGTGACCTGGCC	137
	GTACGACTCACTATAGGGATCCTGAATCTCCTCCACCTGCTTG	
pparγ	AGGTGACACTATAGAATAGAAAGACCCACGGAAACTCA	257
	GTACGACTCACTATAGGGAATGCTCTTGGCGAACTCTGT	
pras40	AGGTGACACTATAGAATAACCAGGGAGAAGGCCATAGT	238
	GTACGACTCACTATAGGGATCACTCCTTCATCCCCTCAC	
raptor	AGGTGACACTATAGAATACTCTTCACCTCCTGCCTCAC	293
	GTACGACTCACTATAGGGAATAAACTGGCCACCAACAGG	
redd1	AGGTGACACTATAGAATAAAGGTCTTCATGGGGGAAAC	162
	GTACGACTCACTATAGGGAACCCGGAGTCTGAGAGATCA	
rictor	AGGTGACACTATAGAATACCTTTTTCTCCCCATTTCGT	234
	GTACGACTCACTATAGGGAGTTTCCTCCGACACATTGGT	
Rxr	AGGTGACACTATAGAATAGCATCTACAAGCCCTGCTTC	147
	GTACGACTCACTATAGGGAGTTCTTCTGGATACTGCGCC	
scd1	AGGTGACACTATAGAATACAGTTGCTGCTGTGTGACCT	276
	GTACGACTCACTATAGGGATGATGTGTTCGTGTGGGACT	
srebp1	AGGTGACACTATAGAATAACCCACAATGGCTACAAAGC	247
	GTACGACTCACTATAGGGACTTGATAAACTGGGGCTGGA	

APPENDIX 4

Table 6: Genes within Fatty Acid Metabolism Targeted in GeXP-Multiplex PCR Analysis

Gene Symbol	Gene Name	Role in Lipid Metabolism	Gene Accession No.	Standard Curve R^2 values Liver	Muscle	Vis. AT
Reference Genes						
β-actin	Beta-actin		NM_001124235	0.9991	0.9743	0.9950
ef1a	Elongation factor 1-alpha		NM_001124339	0.9943	0.9773	0.9945
gapdh	Glyceraldyhyde phosphate dehydrogenase		NM_001124246	0.9937	0.9864	0.9958
rplp2	Acidic ribosomal protein P2		BT074359	0.9994	0.9858	0.9957
Fatty Acid Synthesis						
gpat	Glycerol-3-phosphate acyltransferase	TAG Synthesis	Salem et al., 2012	0.9903	0.9714	0.9887
srebp1	Sterol regulatory element binding protein 1-like	Transcription Factor	Salem et al., 2012	0.9956	0.9538	---
acyl	ATP Citrate Lyase	Fatty Acid Synthesis	Salem et al., 2012	0.9977	0.9966	0.9891
acc	Acetyl-CoA Carboxylase	Fatty Acid Synthesis	Salem et al., 2012	0.9995	0.9631	0.9924
fas	Fatty Acid Synthase	Fatty Acid Synthesis	Salem et al., 2012	0.9960	0.9614	0.9843
scd1	Steroyl-CoA Desaturase (delta-9 desaturase)	Fatty Acid Synthesis	Salem et al., 2012	0.9991	0.9927	0.9834
β-Oxidation						
magl	Monoacylglycerol lipase ABHD12	Lipolysis	EZ770803.1	0.9982	0.9706	0.9985
cpt1a	Carnitine Palmitoyltransferase a	β-oxidation	NM_001124735.1	0.9993	0.9462	0.9979
cpt1b	Carnitine Palmitoyltransferase b	β-oxidation	NM_001171855.1	0.9967	0.9872	0.9860
cpt1c	Carnitine Palmitoyltransferase c	β-oxidation	AJ619768	0.9930	0.9566	0.9969
cpt1d	Carnitine Palmitoyltransferase d	β-oxidation	AJ620356	0.9968	0.9931	0.9926
cpt2	Mitochondrial carnitine palmitoyltransferase I alpha1a	β-oxidation	NM_001246330.1	0.9906	0.9648	0.9912
acat2	Acetyl-CoA acyltransferase 2 (thiolase)	β-oxidation	EZ764956.1	0.9917	0.9755	0.9966
acdh	Acyl CoA DeHydrogenase	β-oxidation	EZ896350.1	---	0.9740	0.9986
acdhm	Medium-chain specific acyl-CoA dehydrogenase	β-oxidation	EZ763374.1	---	0.9162	0.9997
acdhvl	Acyl-CoA dehydrogenase, very long chain	β-oxidation	EZ911051.1	0.9994	0.9503	0.9921
aco	Acetyl-CoA Oxidase	β-oxidation	BX085367	0.9985	0.9845	0.9950
ehhadh	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase/Peroxisomal bifunctional enzyme	β-oxidation	EZ838632 .1	0.9952	0.9777	0.9861
Fatty Acid Transport						
fabp3	Fatty Acid Binding Protein 3	Fatty Acid Transport	NM_001124713	0.9902	0.9826	0.9896
fabp4	Fatty Acid Binding Protein 4 (aP2)	Fatty Acid Transport	JN413683.1	0.9905	0.9915	0.9922
lpl	Lipoprotein Lipase	Fatty Acid Uptake	AJ224693	0.9906	0.9745	0.9950
cd36	Fatty Acid Translocase/Cluster of Differentiation 36	Fatty Acid Uptake	NM_001124511	0.9956	0.9589	0.9917
me	Malic Enzyme	Produces NADPH	Salem et al., 2012	0.9956	0.9642	0.9979
Signaling Factors						
erk	Extracellular signal-regulated kinases	Transcription Factor	QI, 2012	0.8961	0.9861	0.9848
akt2	a serine/threonine-specific protein kinase 2/β	Transcription Factor	Qi, 2012	---	0.9768	0.9930
redd1	REDD1—mTOR1 Repressor	Transcription Factor	Qi, 2012	0.9988	0.9314	0.9990
mo25	MO25—induced by energy stress	Transcription Factor	Qi, 2012	0.9943	0.9840	0.9846
mtor	Mammalian target of rapamycin	Transcription Factor	EU179853	0.9912	0.9660	0.9865
raptor	Raptor	Transcription Factor	Qi, 2012	0.9861	0.9942	0.9883
rictor	Rictor	Transcription Factor	Qi, 2012	0.9957	0.9882	0.9913
pras40	Proline-rich Akt substrate of 40 kilodaltons	Transcription Factor	Salem et al., 2012	0.9941	0.9213	0.9967
gpat	Glycerol-3-phosphate acyltransferase	TAG Synthesis	Salem et al., 2012	0.9903	0.9714	0.9887
pparα	Peroxisome Proliferator-Activated Receptor α	Transcription Factor	NM_001197211.1	---	0.9859	0.9962
pparβ	Peroxisome Proliferator-Activated Receptor β/δ	Transcription Factor	HM536191.1	0.9999	0.9488	0.9842
pparγ	Peroxisome Proliferator-Activated Receptor γ	Transcription Factor	NM_001197212.1	0.9964	0.9834	0.9916
rxr	Retinoid X Receptor gamma variant a	Transcription Factor	NM_001246348.1	0.9993	0.9536	0.9860

APPENDIX 5

Table 7: Fillet fatty acid compositions (% fatty acid) of immature male and female rainbow trout

% FA	Female	Male	p-value
12:0	0.07±0.004	0.07±0.004	0.8275
13:0	0.02±0.001	0.02±0.001	0.3135
14:0	3.44±0.13	3.42±0.13	0.9330
14:1	0.11±0.004	0.10±0.004	0.5233
15:0	0.25±0.01	0.26±0.01	0.8266
16:0	21.74±0.63	22.96±0.63	0.1818
16:1	8.22±0.25	8.17±0.25	0.8787
17:0	0.20±0.01	0.22±0.01	0.1810
17:1	0.09±0.01	0.12±0.01	0.2234
18:0	4.01±0.18[a]	5.01±0.18[b]	**0.0103**
18:1n9	24.97±0.71	26.55±0.71	0.1296
18:2n6	15.54±0.64[b]	13.63±0.64[a]	**0.0464**
18:3n3	1.47±0.08[b]	1.22±0.08[a]	**0.0366**
18:3n6	0.25±0.01	0.22±0.01	0.0976
20:0	0.09±0.01[a]	0.11±0.01[b]	**0.0342**
20:1	1.81±0.11	2.02±0.11	0.2087
20:2	1.67±0.08	1.46±0.008	0.0720
20:3n3	0.09±0.01	0.10±0.01	0.3565
20:3n6	0.62±0.04	0.56±0.04	0.3536
20:4n6	1.46±0.09	1.46±0.10	0.9686
20:5n3	2.98±0.20	2.55±0.20	0.1463
21:0	0.03±0.002	0.03±0.002	0.2689
22:0	0.04±0.01	0.05±0.01	0.2864
22:1n9	0.21±0.03	0.27±0.03	0.1227
22:2	0.73±0.06	0.59±0.06	0.0956
22:6n3	9.47±0.87	8.72±0.87	0.5427
24:1	0.14±0.3[a]	0.21±0.03[b]	**0.0212**
SFA	30.89±0.89	32.78±0.89	0.1441
MUFA	44.01±2.80	49.89±2.80	0.1516
PUFA	48.17±0.03	48.22±0.03	0.3014
ω3	14.37±1.14	12.87±1.14	0.3618
ω6	20.78±0.89	18.25±0.89	0.0592
ω3: ω6	0.69±0.06	0.66±0.06	0.7248
n	16	16	

Values are LSmeans ± SEM reported as mg of fatty acid per g of sample. Means without a common letter represents significant differences between 14-month male and female rainbow trout ($P \leq 0.05$).

APPENDIX 6

Table 8: Fillet fatty acid compositions (% fatty acid) of immature female rainbow trout associated with fillet yield and crude fat content

Fatty Acid (%)	Phenotypic Group				Phenotype
	LY/LF	LY/HF	HY/LF	HY/HF	
12:0	0.09±0.01	0.07±0.01	0.10±0.01	0.07±0.01	0.1812
14:0	0.62±0.02	0.62±0.02	0.63±0.02	0.65±0.02	0.2832
14:1	0.0006±0.0001	0.0005±0.0001	0.0006±0.0001	0.0007±0.0001	0.7396
15:0	0.005±0.0003^{ab}	0.004±0.0003^{a}	0.005±0.0003^{b}	0.004±0.0003^{a}	0.0228
16:0	28.6±0.6	30.2±0.6	29.2±0.6	29.9±0.6	0.3147
16:1	3.5±0.1^{a}	4.2±0.1^{b}	3.3±0.1^{a}	4.1±0.1^{b}	<0.0001
17:0	0.003±0.0001^{b}	0.003±0.0001^{a}	0.004±0.0001^{c}	0.003±0.0001^{a}	<0.0001
18:0	1.4±0.04	1.3±0.04	1.3±0.04	1.4±0.04	0.5134
18:1n-9	40.4±1.0^{a}	44.7±1.0^{b}	39.8±0.9^{a}	44.2±0.9^{b}	0.0002
18:2n-6	16.1±0.5^{c}	12.2±0.5^{a}	16.0±0.5^{c}	13.8±0.5^{b}	<0.0001
20:0	0.001±0.0001	0.001±0.0001	0.001±0.0001	0.001±0.0001	0.3752
18:3n-6	0.004±0.0003	0.004±0.0003	0.004±0.0003	0.003±0.0003	0.2683
20:1	0.35±0.01	0.32±0.01	0.34±0.01	0.33±0.01	0.6541
18:3n-3	0.16±0.01^{b}	0.11±0.01^{a}	0.16±0.01^{b}	0.14±0.01^{b}	<0.0001
20:2	0.11±0.02	0.09±0.02	0.11±0.02	0.08±0.02	0.4953
20:3n-6	0.04±0.002^{b}	0.03±0.002^{b}	0.04±0.002^{b}	0.03±0.002^{a}	0.0332
22:1n-9	0.005±0.0004	0.004±0.0004	0.004±0.0004	0.004±0.0004	0.6425
20:3n-3	0.002±0.0001^{b}	0.001±0.0001^{a}	0.002±0.0001^{ab}	0.002±0.0001^{a}	0.0049
20:4n-6	0.07±0.004^{b}	0.05±0.004^{a}	0.07±0.004^{b}	0.05±0.004^{a}	0.0021
20:5n-3	0.60±0.03^{b}	0.48±0.03^{a}	0.62±0.03^{b}	0.50±0.03^{a}	0.0008
24:1	0.006±0.001^{b}	0.004±0.0005^{a}	0.005±0.005^{ab}	0.004±0.0005^{a}	0.0170
22:6n-3	8.0±0.7^{b}	5.6±0.7^{a}	8.3±0.7^{b}	4.8±0.7^{a}	0.0007
SFA	29.6±0.3^{a}	30.7±0.3^{b}	29.8±0.3^{a}	30.8±0.3^{b}	0.0180
MUFA	35.6±0.5^{a}	38.2±0.5^{b}	35.2±0.4^{a}	38.0±0.4^{b}	<0.0001
PUFA	34.8±0.6^{b}	31.0±0.6^{a}	35.0±0.6^{b}	31.2±0.6^{a}	<0.0001
ω3	15.6±0.5^{b}	13.8±0.5^{a}	16.0±0.5^{b}	13.3±0.5^{a}	<0.0001
ω6	19.2±0.3^{b}	17.3±0.3^{a}	19.0±0.3^{b}	17.9±0.3^{a}	<0.0001
ω3: ω6	0.82±0.03^{b}	0.80±0.03^{b}	0.85±0.03^{b}	0.74±0.03^{a}	0.0301
n	22	22	24	24	

Values are LSMeans ± SEM. Means without a common letter represents significant differences among the four phenotypic groups (P≤0.05). Abbreviations: LY/LF-low yield/low fat; LY/HF-low yield/high fat; HY/LF-high yield/low fat; HY/HF-high yield/high fat.

APPENDIX 7

TROUT GENOME PROJECT: CORRELATIONS WITH LIVER GENE EXPRESSION

[Each table indicates the R^2-value (top number) and p-value (bottom number) for each correlation.]

Table 9: Correlations between Phenotypic Traits and Lipogenic Gene Expression in Liver

	Lipogeneic and Fatty Acid Transport Genes										
	gpat	*srebp1*	*acyl*	*acc*	*fas*	*scd1*	*fabp3*	*fabp4*	*lpl*	*cd36*	*me*
WBW	0.14126	-0.11555	-0.24415	0.17911	0.13032	-0.17120	-0.09845	-0.06053	-0.16554	0.03463	0.34409
	0.2655	0.4777	0.0781	0.1637	0.2970	0.1871	0.4280	0.6517	0.1807	0.7792	**0.0041**
GIT	0.02761	0.07433	0.00034	-0.11270	0.14454	-0.25353	-0.17560	0.20276	-0.23819	0.12277	-0.02659
	0.8285	0.6485	0.9981	0.3831	0.2469	0.0487	0.1552	0.1269	0.0523	0.3186	0.8296
GSI	0.07813	-0.10120	-0.02877	0.15515	0.23207	-0.17318	-0.15296	-0.15358	-0.14793	0.20395	0.16843
	0.5394	0.5344	0.8379	0.2285	0.0608	0.1820	0.2166	0.2497	0.2322	0.0953	0.1698
SepMus	0.03966	-0.35373	-0.22232	0.17107	-0.00363	-0.07398	-0.14180	-0.14089	-0.01791	-0.12516	0.27316
	0.7557	0.0251	0.1096	0.1837	0.9770	0.5710	0.2524	0.2915	0.8856	0.3092	**0.0242**
MusFat	0.01745	-0.23068	-0.29962	0.01581	0.05167	-0.39877	-0.32771	-0.23458	-0.31920	0.05214	0.13370
	0.8911	0.1521	**0.0293**	0.9029	0.6803	**0.0015**	**0.0068**	0.0763	**0.0085**	0.6728	0.2770
n	64	40	53	62	66	61	67	58	67	68	68

Table 10: Correlations between Phenotypic Traits and β-Oxidation Gene Expression in Liver

	β-oxidation Genes											
	magl	*cpt1a*	*cpt1b*	*cpt1c*	*cpt1d*	*cpt2*	*aco*	*acdh*	*acdhm*	*acdhvl*	*ehhadh*	*acat2*
WBW	0.25578	0.32628	-0.02927	0.02831	-0.08213	0.10803	0.15594	.	.	0.29949	0.13703	-0.07360
	0.0448	**0.0066**	0.8695	0.8187	0.7028	0.3955	0.2148	.	.	**0.0171**	0.2726	0.5969
GIT	-0.10178	0.23664	0.14062	0.04790	-0.26628	-0.08983	0.10362	.	.	0.08979	0.16299	-0.17852
	0.4312	0.0520	0.4276	0.6981	0.2085	0.4803	0.4114	.	.	0.4841	0.1910	0.1965
GSI	0.08667	0.34338	-0.17982	0.10139	0.10138	0.06897	0.13047	.	.	0.34882	0.14839	-0.11360
	0.5030	**0.0041**	0.3089	0.4107	0.6374	0.5882	0.3003	.	.	**0.0051**	0.2344	0.4134
SepMus	0.21573	0.14044	0.10749	-0.17052	-0.16489	0.03681	0.09079	.	.	0.20493	-0.01162	-0.07682
	0.0922	0.2533	0.5451	0.1644	0.4413	0.7728	0.4720	.	.	0.1071	0.9262	0.5809
MusFat	-0.04662	0.31958	-0.23695	0.08355	-0.26054	-0.02664	0.10249	.	.	0.18891	-0.00439	-0.00893
	0.7190	**0.0079**	0.1773	0.4982	0.2188	0.8345	0.4165	.	.	0.1381	0.9721	0.9489
n	62	68	34	68	24	64	65	0	0	63	66	54

Table 11: Correlations between Phenotypic Traits and Signaling Factor Gene Expression in Liver

	Transcription Factors											
	erk	*akt2*	*redd1*	*mo25*	*mtor*	*raptor*	*rictor*	*pras40*	*ppar α*	*ppar β*	*ppar γ*	*rxr*
WBW	0.02349	.	0.13406	0.04171	0.03122	0.04174	0.06746	0.13226	.	0.14336	0.05939	0.17382
	0.8649	.	0.2758	0.7356	0.8050	0.7354	0.5875	0.2823	.	0.2623	0.6305	0.1880
GIT	0.07332	.	-0.24132	-0.02180	-0.02441	-0.04988	-0.01588	-0.09269	.	-0.03676	-0.13180	-0.16316
	0.5948	.	0.0474	0.8600	0.8470	0.6862	0.8985	0.4522	.	0.7749	0.2840	0.2169
GSI	-0.03227	.	0.17502	-0.01383	-0.08439	-0.04760	0.05046	-0.00531	.	0.13153	0.05234	0.18089
	0.8151	.	0.1534	0.9108	0.5039	0.6999	0.6851	0.9657	.	0.3042	0.6716	0.1704
SepMus	-0.25261	.	0.16638	-0.05552	-0.01708	-0.04523	-0.05559	0.04164	.	0.08611	0.05086	0.08952
	0.0628	.	0.1751	0.6529	0.8926	0.7142	0.6550	0.7360	.	0.5022	0.6804	0.5001
MusFat	-0.04764	.	0.03058	0.04999	-0.00451	0.13229	0.07244	0.10201	.	0.04512	0.02087	-0.12590
	0.7298	.	0.8045	0.6856	0.9715	0.2822	0.5602	0.4078	.	0.7255	0.8658	0.3420
n	55	0	68	68	65	68	67	68	0	63	68	59

Table 12: Correlations between Fatty Acid Composition and Lipogenic Gene Expression in Liver

	Lipogeneic and Fatty Acid Transport Genes										
	gpat	*srebp1*	*acyl*	*acc*	*fas*	*scd1*	*fabp3*	*fabp4*	*lpl*	*cd36*	*me*
12:0	0.04186	-0.10145	0.07532	0.02332	0.19098	-0.11274	-0.08948	-0.11935	-0.014302	-0.07237	-0.09117
	0.7054	0.4698	0.5295	0.8363	0.0764	0.3225	0.4098	0.3045	0.1837	0.5003	0.3955
14:0	0.00429	0.04791	0.16537	-0.09103	0.03207	0.006465	-0.03885	0.01106	-0.18632	0.0921	-0.13897
	0.9691	0.7333	0.1651	0.419	0.7681	0.9549	0.7209	0.9245	0.0822	0.3907	0.194
14:1	-0.03401	0.0998	0.03328	-0.13505	-0.03783	0.12385	0.08655	0.06748	-0.01245	0.02806	-0.06569
	0.7588	0.4771	0.7814	0.2293	0.7279	0.2769	0.4254	0.5625	0.9084	0.7941	0.5408
15:0	0.02378	-0.1516	0.1814	0.08858	0.1376	-0.09439	-0.18866	-0.05819	-0.07378	-0.1383	-0.27284
	0.83	0.2785	0.1273	0.4317	0.2042	0.408	0.0801	0.6176	0.4945	0.2882	**0.0097**
16:0	-0.02904	0.03245	-0.01805	-0.13335	-0.14711	-0.10266	0.00957	0.09137	-0.06098	0.10755	0.05693
	0.7931	0.8176	0.8804	0.2353	0.1739	0.368	0.9299	0.4324	0.5725	0.3158	0.5962
16:1	-0.03653	0.01587	-0.13006	-0.06861	-0.03993	-0.19547	0.03745	0.09025	-0.1129	0.17089	0.18306
	0.7415	0.9102	0.2762	0.5428	0.7135	0.0843	0.7306	0.4381	0.295	0.1093	0.086
17:0	0.09955	0.11349	0.18687	0.07857	0.05305	0.22301	0.00971	-0.06907	0.12336	-0.01586	-0.00438
	0.3676	0.4184	0.116	0.4857	0.6255	0.0482	0.9289	0.5532	0.2522	0.8827	0.9675
18:0	0.01524	-0.03507	-0.11039	-0.04066	-0.08243	-0.17402	-0.09486	0.00544	-0.12957	-0.06737	-0.1436
	0.8906	0.8032	0.3559	0.7185	0.4478	0.1251	0.3821	0.9628	0.2289	0.5305	0.1794
18:1n-9	0.03082	-0.06856	-0.26963	0.03968	-0.10646	-0.19072	-0.09666	0.00313	-0.08369	0.00438	0.1592
	0.7808	0.6257	0.022	0.725	0.3264	0.0923	0.3731	0.9786	0.4382	0.9675	0.1362
18:2n-6	-0.02643	0.07188	0.37175	0.03961	0.18758	0.22172	0.01493	0.04184	0.02385	-0.04759	-0.32296
	0.8114	0.609	**0.0013**	0.7255	0.0819	**0.0496**	0.8908	0.7197	0.8254	0.6579	**0.002**
20:0	-0.0337	-0.09457	-0.09039	0.00421	0.0159	-0.20212	-0.17396	0.07227	-0.05502	-0.14318	-0.12222
	0.7609	0.5006	0.4502	0.9702	0.8838	0.074	0.1071	0.535	0.6106	0.1807	0.2539
18:3n-6	-0.01961	0.10648	0.12554	0.02719	0.05159	0.25146	0.13558	0.16325	0.22334	-0.02748	-0.1106
	0.8595	0.4479	0.2934	0.8096	0.6351	**0.0254**	0.2105	0.1588	**0.0365**	0.7983	0.3022
20:1	-0.01505	-0.02108	0.18202	-0.00816	0.18636	0.08057	-0.14489	-0.06397	-0.18389	-0.0583	-0.26447
	0.8919	0.8809	0.126	0.9424	0.0839	0.4803	0.1807	0.583	0.0863	0.5874	**0.0123**
18:3n-3	0.06522	0.009	0.29943	0.09953	0.21465	0.11028	-0.03369	0.03762	-0.00249	-0.00898	-0.2788
	0.5556	0.949	**0.0106**	0.3767	**0.0459**	0.3333	0.7567	0.747	0.9817	0.9335	**0.0082**
20:2	-0.01724	-0.10703	0.07616	-0.05673	-0.04519	0.00791	-0.14285	-0.03728	-0.15755	-0.09537	-0.19481
	0.8763	0.4456	0.5249	0.6149	0.6777	0.9448	0.1869	0.7492	0.1426	0.374	0.0673
20:3n-6	-0.01822	0.14135	0.15258	-0.02862	0.10081	0.19665	0.083	-0.08856	0.07806	0.00829	-0.05884
	0.8693	0.3127	0.2007	0.7998	0.3528	0.0824	0.4447	0.4468	0.4698	0.9385	0.5839
22:1n-9	-0.03604	0.06475	0.15165	0.02657	0.09103	0.22705	-0.04239	-0.07857	0.01895	0.0565	-0.0746
	0.7448	0.6451	0.2035	0.8138	0.4017	**0.0442**	0.6966	0.4999	0.8609	0.599	0.4872
20:3n-3	-0.03877	-0.03539	0.19991	0.06146	0.07704	-0.10079	-0.06232	-0.12724	-0.09771	-0.13543	-0.18237
	0.7262	0.8014	0.0923	0.5857	0.4782	0.3768	0.5664	0.2734	0.3651	0.2057	0.0872
20:4n-6	-0.03181	-0.02699	0.06714	-0.0165	0.04879	0.28073	0.206	-0.02994	0.24863	-0.10788	0.02988
	0.7739	0.8479	0.5753	0.8837	6536	**0.0122**	0.0556	0.7974	**0.0195**	0.3143	0.781
20:5n-3	-0.04243	0.05696	0.16566	0.03056	0.12082	0.13859	0.15058	-0.08071	0.11709	-0.06146	0.01581
	0.7015	0.6854	0.1643	0.7866	0.265	0.2232	0.1639	0.4883	0.2773	0.5672	0.8831
24:1	-0.02068	-0.14705	0.10405	-0.01827	0.072	-0.13031	-0.19121	0.00488	-0.07841	-0.1557	-0.28344
	0.8519	0.2934	0.3844	0.8714	0.5075	0.2523	0.0761	0.9666	0.4677	0.1451	**0.0071**
22:6n-3	0.01281	0.02174	0.10183	0.04279	0.12785	0.23611	0.14053	-0.06452	0.2225	-0.09218	-0.00808
	0.9079	0.8772	0.3947	0.7045	0.238	**0.0362**	0.1942	0.5798	**0.0372**	0.3902	0.9401
SAT	-0.02602	0.02833	-0.0187	-0.13271	-0.14249	-0.11153	0.00024	0.08642	-0.07477	0.10022	0.04013
	0.8142	0.8404	0.8761	0.2376	0.188	0.3278	0.9982	0.4579	0.4887	0.3501	0.7088
MUFA	0.02404	-0.06374	-0.27154	0.02793	-0.10425	-0.20787	-0.08912	0.01388	-0.09704	0.02601	0.17223
	0.8281	0.6502	**0.021**	0.8045	0.3366	0.066	0.4117	0.9052	0.3684	0.8088	0.1065
PUFA	-0.00961	0.05317	0.2925	0.04822	0.19023	0.27618	0.09331	-0.0653	0.14364	-0.08531	-0.20175
	0.9309	0.7053	**0.0127**	0.669	0.0776	**0.0138**	0.039	0.5752	0.1818	0.4267	0.058
ω3	0.01087	0.02356	0.10913	0.04366	0.13096	0.23394	0.14147	-0.06527	0.21847	-0.09134	-0.01035
	0.9218	0.867	0.3615	0.6988	0.2266	**0.038**	0.1912	0.5753	**0.0409**	0.3946	0.9233
ω6	-0.02693	0.06849	0.37046	0.03706	0.18456	0.22185	0.01195	-0.04302	0.02072	-0.05059	-0.32493
	0.8079	0.626	**0.0014**	0.7426	0.087	**0.0494**	0.9125	0.7121	0.8481	0.6378	**0.0019**
ω3:ω6	0.00065	-0.0335	-0.08885	0.01387	0.04088	0.12244	0.12483	-0.07042	0.18423	-0.06439	0.15862
	0.9953	0.8118	0.458	0.9022	707	0.2824	0.2493	0.5455	0.0858	0.5489	0.1376
n-value	84	53	72	81	87	79	87	76	88	89	89

Table 13: Correlations between Fatty Acid Composition and β-Oxidation Gene Expression in Liver

	β-oxidation Genes											
	magl	*cpt1a*	*cpt1b*	*cpt1c*	*cpt1d*	*cpt2*	*aco*	*acdh*	*acdhm*	*acdhvl*	*ehhadh*	*acat2*
12:0	-0.05281	-0.0535	0.01063	0.04092	-0.07482	-0.04191	-0.15141	.	.	0.15902	-0.14353	-0.04152
	0.6375	0.3258	0.9441	0.7034	0.6944	0.7051	0.164	.	.	0.151	0.1848	0.7310
14:0	0.04155	277	0.04154	0.19293	0.2823	0.01461	-0.11874	.	.	0.07149	-0.0916	0.4719
	0.7109	0.8395	0.784	0.0701	0.1307	0.8951	0.2762	.	.	0.5207	0.3988	0.6960
14:1	-0.17644	0.02319	-0.18404	0.01406	0.59964	0.02139	-0.21165	.	.	0.01716	-0.18714	0.30805
	0.1128	0.8292	0.2208	0.896	**0.0005**	0.8468	**0.0504**	.	.	0.8776	0.0826	**0.0090**
15:0	0.01826	0.02943	-0.03287	-0.02804	0.05648	0.00375	-0.13141	.	.	0.00918	-0.25084	-0.25217
	0.8707	0.7843	0.8283	0.7943	0.7669	0.973	0.2278	.	.	0.9343	**0.0191**	**0.0339**
16:0	0.0182	0.04389	0.29839	0.01389	0.12826	-0.0526	-0.12443	.	.	0.00686	0.00601	0.06062
	0.8711	0.683	**0.044**	0.8972	0.4994	0.6346	0.2537	.	.	0.9509	0.9559	0.6155
16:1	-0.06379	0.1867	-0.04155	0.09256	-0.12714	-0.07987	0.0475	.	.	0.0697	0.10629	0.21793
	0.5691	0.0798	0.7839	0.3883	0.5032	0.4701	0.6641	.	.	0.5312	0.3272	0.0679
17:0	0.22208	-0.17256	0.10857	0.07048	0.10408	0.13514	-0.06107	.	.	0.01513	-0.13515	-0.11815
	0.0449	0.1059	0.4726	0.5116	0.5841	0.2203	0.5765	.	.	0.892	0.212	0.3264
18:0	-0.06798	0.0712	-0.07737	-0.16429	0.15872	-0.01888	-0.09027	.	.	0.17187	0.02602	-0.14130
	0.544	0.5073	0.6093	0.1239	0.4022	0.8646	0.4085	.	.	0.1203	0.8109	0.2398
18:1n-9	-0.02811	0.15076	-0.28784	-0.11311	-0.09205	-0.02027	0.18129	.	.	0.09094	0.15568	-0.01147
	0.8021	0.1585	0.0524	0.2912	0.6285	0.8548	0.0948	.	.	0.4136	0.1499	0.9244
18:2n-6	0.02318	-0.17258	0.0943	0.12281	0.18324	0.0706	-0.09861	.	.	0.01847	-0.20346	-0.06775
	0.8362	0.1058	0.533	0.2516	0.3324	0.5234	0.3664	.	.	0.8684	0.0587	0.5745
20:0	-0.04393	-0.06754	-0.01201	-0.18815	-0.11162	-0.11635	-0.04892	.	.	0.06717	-0.0418	-0.36739
	0.6951	0.5294	0.9369	0.0774	0.557	0.2919	0.6547	.	.	0.5462	0.7006	**0.0016**
18:3n-6	0.04939	0.05018	0.09572	0.05247	-0.2479	0.15551	0.07461	.	.	-0.0562	0.12718	0.10785
	0.6594	0.6405	0.5269	0.6253	0.1865	0.1578	0.4948	.	.	0.6138	0.2405	0.3707
20:1	-0.06678	0.05684	-0.08274	-0.07408	0.09641	-0.03314	0.03015	.	.	0.12169	-0.04587	-0.35848
	0.5511	0.5968	0.5846	0.4902	0.6123	0.7647	0.7829	.	.	0.2731	0.6731	**0.0021**
18:3n-3	0.04899	-0.12465	0.03665	0.11845	0.34412	0.05701	-0.06743	.	.	0.10315	-0.17261	-0.12238
	0.662	0.2445	0.8089	0.2689	0.0626	0.6065	0.5373	.	.	0.3534	0.1099	0.3093
20:2	-0.06143	0.01736	-0.12971	-0.14401	-0.00776	-0.0265	0.05822	.	.	0.13332	0.02942	-0.33505
	0.5835	0.8717	0.3903	0.1782	0.9675	0.8109	0.5944	.	.	0.2295	0.7868	**0.0043**
20:3n-6	-0.02191	0.15787	0.10431	0.0637	0.13607	0.15868	0.00818	.	.	-0.10018	0.02449	-0.0055
	0.8451	0.1395	0.4903	0.5532	0.4734	0.1494	0.9404	.	.	0.3675	0.8219	0.9637
22:1n-9	-0.02124	-0.11155	-0.21777	-0.0227	0.21927	0.04458	-0.05889	.	.	-0.05881	0.05865	-0.12516
	0.8497	0.298	0.146	0.8328	0.2444	0.6872	0.5902	.	.	0.5974	0.5895	0.2983
20:3n-3	-0.04587	0.02928	0.0135	0.00833	0.19428	0.05091	-0.13463	.	.	-0.03011	-0.11523	-0.19868
	0.6824	0.7853	0.929	0.9382	0.3036	0.6456	0.2165	.	.	0.787	0.2879	0.0967
20:4n-6	0.00712	-0.24218	0.28086	-0.04596	-0.17156	-0.00691	-0.09843	.	.	-0.12337	-0.05362	0.03725
	0.9494	**0.0222**	0.0587	0.6689	0.3647	0.9503	0.3672	.	.	0.2665	0.6218	0.7578
20:5n-3	0.05808	-0.16658	0.34815	0.08984	0.16622	0.10674	-0.19209	.	.	-0.05293	-0.16147	0.09832
	0.6043	0.1187	**0.0177**	0.4024	0.38	0.3339	0.0764	.	.	0.6346	0.1351	0.4146
24:1	-0.04898	0.00051	-0.08382	-0.10122	-0.05218	0.0061	-0.1909	.	.	-0.10814	-0.19434	-0.37134
	0.6621	0.9962	0.5797	0.3453	0.7842	0.9561	0.0956	.	.	0.3305	0.0713	**0.0014**
22:6n-3	0.02522	-0.16133	0.16649	0.03656	-0.09415	0.02724	-0.07735	.	.	-0.21127	-0.0726	-0.00298
	0.8221	0.131	0.2688	0.7338	0.6207	0.8057	0.479	.	.	0.0552	0.504	0.9803
SAT	0.0132	0.04529	0.28514	0.00857	0.14228	-0.05239	-0.13205	.	.	0.02268	0.00202	0.04928
	0.9063	0.6734	0.0548	0.9365	0.4533	0.636	0.2255	.	.	0.8387	0.9852	0.6832
MUFA	-0.03634	0.16895	-0.28384	-0.09644	-0.10366	-0.03057	0.17918	.	.	0.09755	0.16171	0.01501
	0.7459	0.1135	0.0559	0.3686	0.5857	0.7825	0.0988	.	.	0.3803	0.1346	0.9011
PUFA	0.02933	-0.20136	0.15722	0.09489	0.05264	0.06108	-0.10805	.	.	-0.11208	-0.16807	-0.04397
	0.7937	0.0585	0.2967	0.3764	0.7823	0.581	0.322	.	.	0.3131	0.1197	0.7158
ω3	0.02763	-0.16414	0.17646	0.04095	-0.07928	0.03215	-0.08446	.	.	-0.20346	-0.07971	0.00055
	0.8054	0.1243	0.2408	0.7032	0.6771	0.7716	0.4394	.	.	0.0651	0.463	0.9964
ω6	0.02095	-0.17076	0.09132	0.1168	0.17941	0.06951	-0.09602	.	.	0.02123	-0.20013	-0.07835
	0.8518	0.1096	0.5461	0.2757	0.3428	0.5298	0.3792	.	.	0.8489	0.0631	0.5161
ω3:ω6	-0.00864	-0.08477	0.12705	-0.02743	-0.15985	-0.02628	-0.02378	.	.	-0.20741	0.02037	0.02644
	0.9386	0.4296	0.4001	0.7986	0.3988	0.8125	0.8279	.	.	0.0599	0.8514	0.8268
n-value	82	89	46	89	30	84	86	.	.	83	87	71

Table 14: Correlations between Fatty Acid Composition and Signaling Factor Gene Expression in Liver

	Signaling Factors											
	erk	*akt2*	*redd1*	*mo25*	*mtor*	*raptor*	*rictor*	*pras40*	*ppar α*	*ppar β*	*ppar γ*	*rxr*
12:0	-0.05272	.	0.18475	-0.05932	0.02248	-0.0046	-0.09345	-0.06779	.	-0.04807	-0.06392	-0.01843
	0.6647	.	0.083	0.5808	0.8372	0.9659	0.3865	0.5279	.	0.6641	0.5518	0.8719
14:0	-0.00675	.	0.02299	0.03839	0.0351	0.00039	-0.01905	-0.0284	.	-0.04817	-0.06166	-0.04741
	0.9558	.	0.8307	0.721	0.7483	0.9971	0.8601	0.7916	.	0.6635	0.566	0.6782
14:1	-0.05327	.	-0.08646	-0.10787	-0.09617	-0.02144	-0.03208	0.01928	.	-0.3197	-0.05125	-0.10178
	0.6614	.	0.4204	0.3143	0.3784	0.8419	0.7667	0.8577	.	**0.003**	0.6334	0.3721
15:0	-0.12356	.	0.02556	-0.07407	-0.03473	-0.11164	-0.09684	-0.12027	.	0.07687	-0.076	-0.06461
	0.3082	.	0.812	0.4903	0.7509	0.2976	0.3694	0.2616	.	0.4871	0.479	0.5716
16:0	-0.05133	.	-0.10252	0.02074	-0.06813	0.03647	-0.04981	-0.01846	.	-0.02752	0.01931	-0.02617
	0.673	.	0.3391	0.847	0.5331	0.7344	0.6449	0.8637	.	0.8038	0.8575	0.8189
16:1	0.12069	.	-0.11694	0.01464	-0.02722	0.15111	0.04937	0.08466	.	-0.17627	0.00235	-0.07692
	0.3196	.	0.2751	0.8917	0.8035	0.1575	0.6479	0.4302	.	0.1087	0.9826	0.5005
17:0	-0.03294	.	0.27101	0.01369	0.05692	-0.01732	0.02604	-0.01607	.	0.17812	0.04446	0.14201
	0.7866	.	**0.0102**	0.8987	0.6027	0.872	0.8097	0.8812	.	0.105	0.6791	0.2119
18:0	0.06446	.	0.03009	0.04688	-0.0326	-0.03659	-0.08708	-0.07887	.	-0.00382	0.03989	-0.09727
	0.596	.	0.7796	0.6626	0.7657	0.7335	0.4198	0.4625	.	0.9725	0.7105	0.3938
18:1n-9	0.06839	.	-0.03046	0.01987	-0.05034	0.11634	0.10231	0.08511	.	-0.05093	0.10563	-0.08427
	0.5737	.	0.7769	0.8534	0.6453	0.2776	0.3429	0.4277	.	0.6455	0.3245	0.4603
18:2n-6	0.0006	.	0.12524	-0.03825	0.17075	-0.07259	-0.02403	-0.04641	.	0.05837	-0.1559	0.01307
	0.9961	.	0.2422	0.7219	0.116	0.499	0.8241	0.6659	.	0.5979	0.1446	0.909
20:0	-0.0651	.	0.00594	-0.07912	-0.12974	-0.24597	-0.15013	-0.1786	.	0.08824	-0.16009	-0.05153
	0.5924	.	0.9559	0.4611	0.2338	**0.0202**	0.1627	0.094	.	0.4248	0.134	0.652
18:3n-6	-0.058	.	0.04575	0.20756	0.15431	0.08024	0.01413	0.01998	.	0.02738	0.07502	0.10666
	0.6334	.	0.6703	0.051	0.156	0.4548	0.8961	0.8525	.	0.8047	0.4848	0.3495
20:1	-0.02641	.	0.02299	0.0883	0.08964	-0.22407	-0.105	-0.13573	.	0.10077	-0.16541	-0.04264
	0.8282	.	0.8306	0.4106	0.4118	**0.0349**	0.3303	0.2047	.	0.3617	0.1214	0.7091
18:3n-3	-0.00554	.	0.07301	-0.13211	0.15637	-0.13307	-0.00971	-0.03746	.	0.09121	-0.12714	-0.0228
	0.9637	.	0.4965	0.2171	0.1505	0.2138	0.9284	0.7275	.	0.4093	0.2351	0.8419
20:2	-0.13148	.	-0.09544	0.0735	-0.01165	-0.16404	-0.04327	-0.11231	.	0.11546	-0.04785	-0.03116
	0.2779	.	0.3736	0.4937	0.9152	0.1245	0.689	0.2947	.	0.2956	0.6561	0.7851
20:3n-6	-0.0649	.	-0.05832	0.28311	0.07008	-0.08564	-0.02009	-0.00768	.	0.11218	0.09118	0.13711
	0.5935	.	0.5872	**0.0072**	0.5214	0.4249	0.8526	0.9431	.	0.3097	0.3954	0.2282
22:1n-9	0.00565	.	0.02313	-0.01374	0.10694	-0.12305	0.04031	0.00508	.	0.05602	-0.15371	-0.016
	0.963	.	0.8296	0.8983	0.3271	0.2506	0.7092	0.9623	.	0.6128	0.1504	0.8887
20:3n-3	0.01435	.	0.01892	-0.18492	0.00329	-0.20729	0.0002	-0.02751	.	0.0393	-0.10208	-0.12189
	0.9062	.	0.8603	0.0828	0.976	0.0513	0.9985	0.798	.	0.7227	0.3412	0.2846
20:4n-6	-0.10827	.	0.12478	-0.00585	-0.06924	-0.13362	-0.11504	-0.10262	.	-0.02642	-0.03252	0.11377
	0.3723	.	0.244	0.9566	0.5264	0.2119	0.2858	0.3386	.	0.8115	0.7623	0.3181
20:5n-3	-0.05579	.	0.07234	-0.05449	-0.04051	-0.13842	-0.10874	-0.07239	.	0.00195	-0.05311	0.12267
	0.6464	.	0.5005	0.612	0.7112	0.1958	0.3132	0.5002	.	0.9859	0.6211	0.2815
24:1	-0.14047	.	0.00707	-0.15604	-0.03865	-0.23466	-0.18483	-0.2067	.	0.06269	-0.15383	-0.02157
	0.2461	.	0.9476	0.1442	0.7239	**0.0269**	0.0847	0.052	.	0.5694	0.1501	0.8503
22:6n-3	-0.0863	.	0.04169	-0.02199	-0.01926	-0.17283	-0.09553	-0.08097	.	0.09042	-0.02926	0.17129
	0.4775	.	0.6981	0.838	0.8603	0.1053	0.376	0.4507	.	0.4134	0.7855	0.1321
SAT	-0.04638	.	-0.09255	0.02273	-0.06651	0.03265	-0.05611	-0.02508	.	-0.02882	0.01825	-0.03315
	0.703	.	0.3883	0.8326	0.5429	0.7613	0.6036	0.8155	.	0.7947	0.8652	0.7718
MUFA	0.07978	.	-0.04419	0.02191	-0.05039	0.12772	0.10251	0.09041	.	-0.07059	0.09861	-0.09125
	0.5115	.	0.6809	0.8385	0.645	0.233	0.3419	0.3995	.	0.5234	0.3579	0.4238
PUFA	-0.05646	.	0.09967	-0.03593	0.08984	-0.1513	-0.0737	-0.07902	.	0.08967	-0.11276	0.11146
	0.6425	.	0.3527	0.7382	0.4107	0.157	0.495	0.4617	.	0.4173	0.2928	0.3281
ω3	-0.08537	.	0.04441	-0.02544	-0.01858	-0.17382	-0.09692	-0.08151	.	0.08761	-0.03223	.169.69
	0.4823	.	0.6794	0.813	0.8652	0.1032	0.369	0.4476	.	0.4281	0.7643	0.1349
ω6	-0.00429	.	0.1213	-0.03432	0.1681	-0.07787	-0.02587	-0.04995	.	0.06148	-0.1552	0.01327
	0.9719	.	0.2475	0.7495	0.1218	0.4682	0.8109	0.642	.	0.5785	0.1464	0.9076
ω3:ω6	-0.09937	.	-0.04489	-0.00346	-0.11948	-0.17092	-0.0909	-0.08348	.	0.04554	0.02533	0.15346
	0.4131	.	0.6761	0.9743	0.2732	0.1093	0.3996	0.4367	.	0.6808	0.8137	0.1769
n-value	70	.	89	89	86	89	88	89	.	84	89	79

APPENDIX 8

TROUT GENOME PROJECT: CORRELATIONS WITH MUSCLE GENE EXPRESSION

[Each table indicates the R^2-value (top number) and p-value (bottom number) for each correlation.]

Table 15: Correlations between Phenotypic Traits and Lipogenic Gene Expression in Muscle

	Lipogeneic and Fatty Acid Transport Genes										
	gpat	*srebp1*	*acyl*	*acc*	*fas*	*scd1*	*fabp3*	*fabp4*	*lpl*	*cd36*	*me*
WBW	-0.07619	0.14529	-0.08167	0.03562	-0.00940	-0.05186	-0.00520	0.06138	0.00758	0.01312	0.05375
	0.4909	0.6054	0.5145	0.7522	0.9324	0.7150	0.9647	0.6034	0.9514	0.9057	0.6272
GIT	-0.04937	-0.27197	-0.00313	-0.04175	0.01614	0.15690	0.08466	0.14061	0.00979	0.23407	0.06398
	0.6556	0.3268	0.9801	0.7113	0.8842	0.2666	0.4702	0.2321	0.9373	0.0321	0.5631
GSI	-0.02732	0.34405	-0.05468	-0.08833	-0.01775	-0.06176	-0.15201	0.06899	-0.08924	-0.16268	-0.01601
	0.8063	0.2092	0.6653	0.4359	0.8734	0.6636	0.1960	0.5619	0.4727	0.1417	0.8858
SepMus	-0.07264	0.07722	-0.16230	0.00574	-0.01024	-0.05887	0.02217	0.01393	0.12082	-0.13386	-0.14437
	0.5114	0.7844	0.1929	0.9594	0.9263	0.6785	0.8502	0.9062	0.3301	0.2248	0.1901
Trim	0.02769	0.14001	0.02219	-0.00848	-0.03134	-0.06036	-0.07652	-0.10138	-0.05011	-0.03624	0.01913
	0.8025	0.6187	0.8596	0.9401	0.7772	0.6708	0.5141	0.3901	0.6872	0.7434	0.8629
BF	-0.05487	0.09409	-0.07430	0.10711	0.01480	0.07070	0.07606	0.00890	0.11379	0.00331	0.04970
	0.6201	0.7387	0.5532	0.3412	0.8937	0.6185	0.5166	0.9400	0.3592	0.9761	0.6535
Cook	-0.14195	-0.32701	0.00035	-0.16370	-0.11654	-0.03410	-0.03420	-0.21531	-0.05883	-0.15153	-0.15487
	0.2033	0.2538	0.9978	0.1494	0.2971	0.8122	0.7739	0.0693	0.6363	0.1742	0.1648
Force	0.18957	0.16907	0.05023	0.11445	0.18033	0.12515	0.05010	0.05696	-0.16094	0.10577	0.15103
	0.0841	0.5469	0.6888	0.3090	0.1007	0.3767	0.6695	0.6298	0.1932	0.3383	0.1703
Fat	0.01441	-0.03412	-0.04577	0.07601	0.05063	-0.12710	-0.05302	0.00400	0.11996	0.11037	0.11279
	0.8965	0.9039	0.7152	0.5000	0.6474	0.3692	0.6514	0.9730	0.3336	0.3176	0.3070
n-value	84	15	66	81	84	52	75	74	67	84	84

Table 16: Correlations between Phenotypic Traits and β-Oxidation Gene Expression in Muscle

	β-oxidation Genes											
	magl	*cpt1a*	*cpt1b*	*cpt1c*	*cpt1d*	*cpt2*	*aco*	*acdh*	*acdhm*	*acdhvl*	*ehhadh*	*acat2*
WBW	0.09015	0.05073	-0.11638	0.15986	-0.11008	0.10138	0.04073	.	.	0.08889	-0.01203	-0.08507
	0.4148	0.6468	0.2918	0.1463	0.3573	0.3618	0.7130	.	.	0.4330	0.9135	0.4417
GIT	0.06429	0.09046	-0.09814	0.08409	0.02612	0.02132	0.01538	.	.	-0.03744	0.15095	0.09165
	0.5612	0.4132	0.3745	0.4470	0.8276	0.8483	0.8896	.	.	0.7416	0.1705	0.4070
GSI	0.04856	-0.05646	-0.06759	-0.01837	-0.04696	-0.10345	-0.03957	.	.	-0.05751	-0.15301	-0.08867
	0.6628	0.6122	0.5438	0.8691	0.6973	0.3550	0.7224	.	.	0.6147	0.1673	0.4253
SepMus	0.03574	0.02255	-0.14882	-0.02125	-0.24546	0.01583	0.00483	.	.	0.10188	-0.06104	-0.12489
	0.7469	0.8387	0.1767	0.8479	0.0377	0.8871	0.9653	.	.	0.3685	0.5812	0.2577
Trim	-0.09079	-0.06205	0.10665	-0.06163	0.08269	-0.08347	0.00116	.	.	-0.05278	-0.01499	-0.02633
	0.4115	0.5750	0.3342	0.5776	0.4898	0.4531	0.9917	.	.	0.6419	0.8924	0.8121
BF	0.00941	0.06268	-0.09414	0.19380	-0.08646	0.03419	0.07144	.	.	0.15831	0.01481	-0.02753
	0.9323	0.5711	0.3943	0.0773	0.4702	0.7590	0.5184	.	.	0.1608	0.8936	0.8037
Cook	-0.14358	-0.18325	-0.08993	-0.13763	-0.07729	-0.21028	-0.22203	.	.	-0.26278	-0.06149	-0.14777
	0.1981	0.0994	0.4217	0.2176	0.5248	0.0595	0.0450	.	.	0.0201	0.5832	0.1852
Force	0.20655	0.31823	0.03204	0.20766	-0.29300	0.21932	0.18931	.	.	0.11081	0.16118	0.07888
	0.0594	0.0032	0.7724	0.0580	0.0125	0.0464	0.0846	.	.	0.3278	0.1430	0.4757
Fat	0.06043	0.13825	-0.08037	0.24715	-0.10243	0.05862	0.06228	.	.	0.09215	0.04070	-0.01957
	0.5851	0.2098	0.4674	0.0234	0.3919	0.5986	0.5736	.	.	0.4162	0.7132	0.8597
n-value	84	84	84	84	72	83	84	0	0	80	84	84

Table 17: Correlations between Phenotypic Traits and Signaling Factor Gene Expression in Muscle

	Signaling Factors											
	erk	*akt2*	*redd1*	*mo25*	*mtor*	*raptor*	*rictor*	*pras40*	*ppar α*	*ppar β*	*ppar γ*	*rxr*
WBW	0.00886	0.18124	-0.19845	-0.08242	0.01354	-0.07286	0.01259	-0.08815	.	-0.08127	-0.09912	0.09319
	0.9370	0.5180	0.0704	0.4561	0.9027	0.7886	0.9106	0.4253	.	0.4624	0.6528	0.4021
GIT	0.13625	0.15357	-0.14106	0.05613	0.10926	0.04393	-0.03642	-0.00571	.	-0.05023	-0.14878	0.10488
	0.2223	0.5848	0.2006	0.6121	0.3225	0.8717	0.7453	0.9589	.	0.6500	0.4981	0.3454
GSI	-0.01707	0.19384	-0.16552	-0.06883	-0.11189	-0.13530	-0.11582	-0.14849	.	-0.07120	-0.18238	-0.13770
	0.8798	0.4888	0.1348	0.5364	0.3139	0.6174	0.3032	0.1803	.	0.5224	0.4049	0.2173
SepMus	0.03113	-0.04251	-0.11016	-0.27087	-0.09423	-0.43944	-0.07623	-0.20468	.	-0.08002	0.13717	-0.06233
	0.7813	0.8804	0.3185	0.0127	0.3939	0.0886	0.4961	0.0618	.	0.4693	0.5326	0.5756
Trim	-0.14717	-0.04324	0.20656	0.07691	-0.04168	0.05531	0.03117	0.11796	.	0.10295	0.03241	-0.04560
	0.1870	0.8784	0.0594	0.4868	0.7066	0.8388	0.7810	0.2852	.	0.3514	0.8833	0.6823
BF	-0.03038	0.21019	-0.24583	-0.14323	0.02040	-0.17596	0.07307	-0.06680	.	0.01212	-0.10751	0.04850
	0.7864	0.4521	0.0242	0.1937	0.8538	0.5145	0.5142	0.5460	.	0.9129	0.6254	0.6633
Cook	-0.13073	0.08558	0.11770	-0.02210	-0.06217	-0.03908	-0.07280	-0.01693	.	-0.07482	-0.16436	-0.20363
	0.2477	0.7617	0.2923	0.8438	0.5790	0.8900	0.5210	0.8800	.	0.5041	0.4648	0.0683
Force	0.15444	-0.20593	-0.03588	0.11134	0.15088	-0.18197	0.07837	-0.05481	.	0.15692	0.11348	0.09952
	0.1659	0.4615	0.7459	0.3133	0.1707	0.5000	0.4840	0.6205	.	0.1540	0.6062	0.3707
Fat	0.05634	0.19420	-0.19681	-0.02368	0.20311	-0.11606	0.08793	-0.01833	.	0.01529	-0.17009	0.11622
	0.6152	0.4880	0.0728	0.8307	0.0639	0.6686	0.4321	0.8686	.	0.8902	0.4378	0.2954
n-value	82	15	84	84	84	16	82	84	0	84	23	83

Table 18: Correlations between Fatty Acid Composition and Lipogenic Gene Expression in Muscle

	Lipogenic and Fatty Acid Gene Expression										
	gpat	*srebp1*	*acyl*	*acc*	*fas*	*scd1*	*fabp3*	*fabp4*	*lpl*	*cd36*	*me*
12:0	-0.07162	-0.30081	0.18451	-0.01340	-0.04084	0.14513	-0.00022	0.10639	-0.13817	-0.03073	-0.00580
	0.5174	0.2760	0.1381	0.9055	0.7123	0.3046	0.9985	0.3670	0.2648	0.7814	0.9583
14:0	-0.04108	-0.08005	-0.02099	0.10353	0.08370	-0.09798	0.10098	0.13130	0.09454	0.02773	0.08310
	0.7106	0.7767	0.8671	0.3577	0.4490	0.4895	0.3886	0.2648	0.4467	0.8023	0.4523
14:1	-0.05367	-0.22508	-0.20319	-0.01371	-0.03472	-0.20324	-0.03091	-0.00631	0.14535	-0.15506	-0.09233
	0.6278	0.4199	0.1018	0.9033	0.7538	0.1484	0.7924	0.9574	0.2406	0.1590	0.4035
15:0	-0.03695	0.19424	0.09262	-0.06868	-0.04552	0.03205	0.02414	0.09734	0.00767	0.09410	0.01638
	0.7386	0.4879	0.4595	0.5424	0.6810	0.8216	0.8371	0.4093	0.9509	0.3946	0.8824
16:0	-0.07530	-0.19970	-0.01757	0.07632	0.10213	-0.22603	-0.03296	-0.02411	-0.01603	-0.02122	0.00481
	0.4960	0.4755	0.8887	0.4983	0.3553	0.1071	0.7789	0.8385	0.8975	0.8481	0.9654
16:1	0.00408	-0.34514	-0.03350	0.07367	0.07367	-0.11508	-0.00083	0.07533	0.18233	-0.00931	0.02539
	0.9706	0.2077	0.7894	0.5134	0.5054	0.4166	0.9943	0.5235	0.1397	0.9330	0.8187
17:0	-0.04213	0.30185	-0.05040	0.06246	0.01903	0.00798	0.02172	0.00378	-0.25076	-0.06814	-0.02874
	0.7036	0.2742	0.6878	0.5796	0.8636	0.9552	0.8532	0.9745	**0.0407**	0.5380	0.7952
18:0	0.04567	0.19779	0.16635	0.08754	0.03868	-0.03522	-0.17386	-0.11302	-0.31359	0.16227	0.16478
	0.6800	0.4798	0.1819	0.4371	0.7268	0.8042	0.1358	0.3377	**0.0098**	0.1403	0.1342
18:1n-9	0.10973	-0.01680	0.06301	0.15174	0.10015	-0.06162	-0.15962	-0.06500	0.08218	0.18605	0.18970
	0.3204	0.9526	0.6152	0.1763	0.3647	0.6643	0.1713	0.5822	0.5085	0.0902	0.0839
18:2n-6	0.01965	0.21684	0.09161	-0.15835	-0.08522	0.31367	0.29136	0.16899	-0.02396	-0.12982	-0.03895
	0.8592	0.4376	0.4644	0.1580	0.4409	**0.0236**	**0.0112**	0.1500	0.8474	0.2392	0.7250
20:0	-0.02448	0.12882	0.07149	-0.08959	0.00140	0.13984	0.00701	-0.13821	-0.00454	0.06954	0.02802
	0.8250	0.6473	0.5684	0.4264	0.9899	0.3228	0.9524	0.2403	0.9709	0.5296	0.8003
18:3n-6	-0.08800	0.02264	0.04422	-0.13578	-0.09460	0.02827	-0.07376	-0.19074	0.09023	-0.15862	-0.06019
	0.4260	0.9362	0.7244	0.2268	0.3920	0.8423	0.5294	0.1035	0.4678	0.1496	0.5865
20:1	0.04333	0.49183	0.20925	-0.17451	-0.07284	0.29698	0.16165	0.08138	-0.07629	0.09792	0.07695
	0.6955	0.0626	0.0918	0.1192	0.5102	**0.0325**	0.1659	0.4907	0.5395	0.3755	0.4866
18:3n-3	0.13154	0.35188	0.05677	-0.01731	0.01721	0.26255	0.35916	0.34179	0.08090	0.03312	0.05474
	0.2330	0.1984	0.6507	0.8781	0.8765	0.0601	**0.0016**	**0.0029**	0.5152	0.7648	0.6209
20:2	-0.07500	0.59914	-0.00738	-0.13901	-0.11768	0.03493	-0.04357	-0.11207	-0.04878	0.03363	0.02307
	0.4977	**0.0183**	0.9531	0.2158	0.2864	0.8058	0.7105	0.3418	0.6950	0.7613	0.8350
20:3n-6	-0.07482	0.70171	0.15636	-0.16397	-0.14396	0.03813	0.05291	-0.18917	0.10676	-0.16216	-0.03066
	0.4988	**0.0035**	0.2099	0.1435	0.1914	0.7884	0.6521	0.1065	0.3898	0.1406	0.7819
22:1n-9	0.01659	0.13138	-0.02557	-0.09320	-0.04647	-0.04646	0.14210	0.11763	-0.08923	0.00216	-0.09318
	0.8810	0.6407	0.8385	0.4079	0.6747	0.7436	0.2239	0.3182	0.4727	0.9844	0.3992
20:3n-3	0.15461	0.58062	0.16313	-0.01726	0.02957	0.19195	0.23262	0.14488	0.04944	-0.02098	0.13940
	0.1630	**0.0232**	0.1906	0.8785	0.7895	0.1728	**0.0446**	0.2181	0.6911	0.8497	0.2060
20:4n-6	-0.03836	-0.26207	-0.17335	-0.01502	-0.15483	0.03090	-0.03723	-0.03833	-0.07185	-0.09977	-0.23407
	0.7290	0.3454	0.1639	0.1808	0.1597	0.8278	0.7512	0.7457	0.5634	0.3666	**0.0321**
20:5n-3	-0.05652	-0.38107	-0.09724	-0.13105	-0.12374	-0.07153	-0.04400	0.08264	-0.11879	-0.01324	-0.19661
	0.6069	0.1611	0.4373	0.2435	0.2621	0.6143	0.7078	0.4869	0.3383	0.2299	0.0730
24:1	-0.05211	0.28319	0.04058	-0.18508	-0.12427	0.01453	-0.05939	-0.03842	-0.16647	0.02450	-0.13321
	0.6378	0.3064	0.7463	0.0981	0.2600	0.9186	0.6128	0.7452	0.1742	0.8250	0.2271
22:6n-3	-0.10191	-0.01516	-0.13678	-0.14798	-0.15840	0.01298	0.01982	-0.04251	-0.07556	-0.13394	-0.23927
	0.3563	0.9572	0.2735	0.1874	0.1501	0.9272	0.8660	0.7191	0.5434	0.2245	**0.0284**
SAT	-0.07171	-0.18464	-0.00209	0.08067	0.10159	-0.22053	-0.03985	-0.02448	-0.03681	-0.00990	0.01706
	0.5168	0.5101	0.9867	0.4741	0.3578	0.1162	0.7343	0.8360	0.7674	0.9287	0.8776
MUFA	0.10445	-0.04463	0.05598	0.15001	0.10329	-0.06764	-0.14846	-0.05127	0.09784	0.17527	0.18300
	0.3444	0.8745	0.6553	0.1813	0.3498	0.6338	0.2037	0.6644	0.4309	0.1108	0.0957
PUFA	-0.05777	0.11501	-0.04942	-0.18501	-0.15337	0.16481	0.16538	0.06205	-0.06663	-0.15855	-0.18083
	0.6017	0.6832	0.6935	0.0982	0.1637	0.2430	0.1562	0.5995	0.5921	0.1497	0.0997
ω3	-0.09955	-0.03052	-0.13604	-0.14859	-0.15804	0.01221	0.02090	-0.03455	-0.07714	-0.13458	-0.23881
	0.3676	0.9140	0.2761	0.1855	0.1511	0.9316	0.8588	0.7701	0.5349	0.2223	**0.0287**
ω6	0.01653	0.22119	0.08949	-0.16238	-0.08939	0.31127	0.28601	0.16339	-0.02518	-0.12854	-0.03955
	0.8814	0.4282	0.4749	0.1475	0.4187	**0.0247**	**0.0129**	0.1642	0.8397	0.2439	0.7209
ω3:ω6	-0.10785	-0.20920	-0.17458	-0.10432	-0.14497	-0.09454	-0.09724	-0.10909	-0.05346	-0.11441	-0.25022
	0.3288	0.4543	0.1609	0.3540	0.1883	0.5050	0.4065	0.6549	0.6674	0.3001	**0.0217**
n-value	84	15	66	81	84	52	75	74	67	84	84

Table 19: Correlations between Fatty Acid Composition and β-Oxidation Gene Expression in Muscle

	β-oxidation Genes											
	magl	*cpt1a*	*cpt1b*	*cpt1c*	*cpt1d*	*cpt2*	*aco*	*acdh*	*acdhm*	*acdhvl*	*ehhadh*	*acat2*
12:0	-0.04632	0.03890	0.15397	-0.02047	0.07779	0.03596	-0.04477	.	.	0.07902	0.12405	0.03664
	0.6757	0.7253	0.1620	0.8552	0.5160	0.7469	0.6860	.	.	0.4860	0.2609	0.7407
14:0	0.05073	0.08306	0.05469	0.18382	0.00773	0.09727	0.14076	.	.	0.22832	0.19502	0.01332
	0.6468	0.4526	0.6212	0.0983	0.9486	0.3817	0.2015	.	.	**0.0416**	0.0754	0.9043
14:1	-0.09427	-0.10596	-0.10098	-0.05934	-0.18525	-0.18455	-0.02650	.	.	-0.00101	-0.13437	-0.15158
	0.3937	0.3374	0.3607	0.5964	0.1192	0.0949	0.8109	.	.	0.9929	0.2230	0.1687
15:0	0.01438	-0.05765	0.16116	-0.10845	0.12781	0.08105	0.03273	.	.	0.00602	0.09744	0.11720
	0.8967	0.6024	0.1431	0.3321	0.2847	0.4664	0.7676	.	.	0.9577	0.3779	0.2884
16:0	0.06975	0.03563	-0.08986	0.13064	-0.10834	-0.05542	0.05522	.	.	0.16093	0.09728	-0.03625
	0.5284	0.7476	0.4163	0.2421	0.3650	0.6188	0.6178	.	.	0.1538	0.3787	0.7434
16:1	0.00534	0.01173	-0.05361	0.16997	0.02345	-0.12010	0.00532	.	.	0.05863	0.07992	-0.00048
	0.9616	0.9156	0.6281	0.1268	0.8450	0.2795	0.0962	.	.	0.6055	0.4699	0.9966
17:0	0.02325	-0.00814	0.05716	-0.11005	-0.03107	0.09478	0.06662	.	.	0.06853	-0.07396	-0.01451
	0.8337	0.9414	0.6055	0.3250	0.7956	0.3940	0.5471	.	.	0.5458	0.5037	0.8958
18:0	0.06842	0.16199	0.10832	0.03163	-0.04114	0.16808	0.12019	.	.	0.12038	0.06679	0.11023
	0.5363	0.1410	0.3267	0.7778	0.7315	0.1288	0.2761	.	.	0.2875	0.5461	0.3182
18:1n-9	0.04368	0.16321	0.02506	0.08914	-0.10508	0.06959	0.15743	.	.	0.06390	-0.01472	0.07906
	0.6932	0.1380	0.8210	0.4258	0.3797	0.5319	0.1527	.	.	0.5733	0.8942	0.4747
18:2n-6	-0.04814	-0.04102	0.18791	-0.12813	0.24211	0.10673	-0.05717	.	.	-0.07955	0.07392	0.05514
	0.6637	0.7110	0.0870	0.2513	**0.0405**	0.3369	0.6055	.	.	0.4830	0.5040	0.6184
20:0	0.02631	0.06809	-0.04848	-0.10902	-0.15426	0.06381	0.04319	.	.	-0.06445	0.10460	-0.00436
	0.8122	0.5383	0.6614	0.3296	0.1957	0.5666	0.6965	.	.	0.5700	0.3437	0.9686
18:3n-6	-0.08190	-0.10960	0.11863	-0.22311	-0.03739	-0.10260	-0.12447	.	.	-0.18563	-0.04286	-0.08533
	0.4589	0.3210	0.2825	**0.0439**	0.7552	0.3560	0.2593	.	.	0.0992	0.6987	0.4403
20:1	0.05649	0.01879	0.20079	-0.17691	0.16334	0.17325	0.05771	.	.	-0.07929	0.20296	0.21698
	0.6098	0.8653	0.0670	0.1118	0.1704	0.1173	0.6021	.	.	0.4845	0.0641	**0.0474**
18:3n-3	0.02779	0.06886	0.25585	-0.03263	0.20861	0.21871	0.13219	.	.	0.12683	0.24875	0.16854
	0.8019	0.5337	**0.0188**	0.7710	0.0787	**0.0470**	0.2307	.	.	0.2623	**0.0225**	0.1254
20:2	-0.04566	-0.10263	0.00677	-0.19876	-0.04139	-0.05059	-0.01901	.	.	-0.13295	0.06758	0.01829
	0.6800	0.3529	0.9513	0.0734	0.7300	0.6497	0.8637	.	.	0.2397	0.5413	0.8688
20:3n-6	-0.08324	-0.24224	0.14943	-0.23974	0.15175	-0.08575	-0.01449	.	.	-0.23115	-0.14162	-0.03163
	0.4516	**0.0264**	0.1479	**0.0301**	0.2032	0.4408	0.1885	.	.	**0.0391**	0.1988	0.7751
22:1n-9	-0.01307	-0.21030	-0.00755	-0.06993	-0.06866	-0.01101	-0.06368	.	.	0.03985	0.04302	0.11574
	0.9061	0.0548	0.9457	0.5325	0.5666	0.9213	0.5649	.	.	0.7256	0.6976	0.2944
20:3n-3	0.08560	0.01085	0.28813	-0.00266	0.19904	0.21525	0.11347	.	.	0.12351	-0.02573	0.18351
	0.4388	0.9220	**0.0079**	0.9811	0.0937	**0.0507**	0.3041	.	.	0.2750	0.8163	0.0947
20:4n-6	-0.06128	-0.14397	-0.14016	-0.17978	-0.07888	-0.13333	-0.18780	.	.	-0.16103	-0.10349	-0.01306
	0.5797	0.1914	0.2035	0.1061	0.5101	0.2295	0.0871	.	.	0.1536	0.3488	0.2363
20:5n-3	-0.10822	-0.14701	-0.00353	-0.10038	0.01477	-0.05438	-0.15626	.	.	-0.09726	-0.08273	-0.08254
	0.3271	0.1820	0.9746	0.3696	0.9020	0.6254	0.1558	.	.	0.3907	0.4543	0.4554
24:1	-0.05284	-0.16433	-0.00534	-0.16418	0.06193	-0.01611	-0.12656	.	.	-0.17665	-0.04298	0.02261
	0.6331	0.1352	0.9615	0.1405	0.6053	0.8851	0.2513	.	.	0.1170	0.6978	0.8382
22:6n-3	-0.07808	0.22257	-0.11810	-0.14878	0.03734	-0.12461	-0.21740	.	.	-0.16967	-0.14936	-0.13139
	0.4802	**0.0419**	0.2847	0.1822	0.7555	0.2617	**0.0470**	.	.	0.1324	0.1751	0.2335
SAT	0.07155	0.04722	-0.07470	0.13136	-0.10432	-0.03916	0.06330	.	.	0.16915	0.10489	-0.02664
	0.5178	0.6697	0.4995	0.2394	0.3832	0.7252	0.5673	.	.	0.1336	0.3423	0.8099
MUFA	0.04254	0.15542	0.01888	0.10447	-0.09429	0.05154	0.14968	.	.	0.06714	-0.00062	0.07716
	0.7008	0.1580	0.8647	0.3503	0.4308	0.6436	0.1742	.	.	0.5540	0.9955	0.4854
PUFA	-0.07974	-0.17184	0.02401	-0.17099	0.14828	-0.02629	-0.17543	.	.	-0.15680	-0.05794	-0.05738
	0.4709	0.1180	0.8284	0.1245	0.2139	0.8135	0.1104	.	.	0.1648	0.6006	0.6042
ω3	-0.07960	-0.22055	-0.01118	-0.14829	0.03866	-0.12057	-0.21528	.	.	-0.16679	-0.14539	-0.12874
	0.4717	**0.0438**	0.3111	0.1837	0.7471	0.2776	**0.0492**	.	.	0.1392	0.1870	0.2432
ω6	-0.04970	-0.04561	0.18535	-0.13481	0.23743	0.10250	-0.05896	.	.	-0.08468	0.07374	0.05391
	0.6535	0.6804	0.0914	0.2272	**0.0446**	0.3565	0.5942	.	.	0.4552	0.5050	0.6262
ω3:ω6	-0.08130	-0.22857	-0.19178	-0.11829	-0.05809	-0.18413	-0.22166	.	.	-0.16775	-0.20296	-0.17828
	0.4622	**0.0365**	0.0805	0.2899	0.6279	0.0956	**0.0427**	.	.	0.1369	0.0641	0.1047
n-value	84	84	84	82	72	83	84	.	.	80	84	84

Table 20: Correlations between Fatty Acid Composition and Signaling Factor Gene Expression in Muscle

	Signaling Factors											
	erk	*akt2*	*redd1*	*mo25*	*mtor*	*raptor*	*rictor*	*pras40*	*ppar α*	*ppar β*	*ppar γ*	*rxr*
12:0	-0.02424	0.19209	0.30456	0.05605	0.01179	0.35833	0.06519	0.09894	.	0.00719	0.18350	0.03160
	0.8289	0.4928	**0.0049**	0.6126	0.9152	0.1729	0.5656	0.3735	.	0.9483	0.4020	0.7767
14:0	0.11190	-0.08003	0.04599	-0.03137	0.14377	-0.17190	0.01055	0.04680	.	0.18472	-0.04886	0.23090
	0.3169	0.7768	0.6778	0.7770	0.1920	0.5244	0.9260	0.6744	.	0.0925	0.8248	**0.0357**
14:1	-0.05338	0.24173	-0.08050	-0.12974	-0.01737	-0.42962	-0.13824	-0.13040	.	0.04962	-0.13322	-0.05587
	0.6338	0.3854	0.4666	0.2395	0.8754	0.0968	0.2214	0.2400	.	0.6540	0.5445	0.6159
15:0	-0.07123	0.47412	0.25515	0.08635	-0.10364	0.27174	-0.00492	0.12920	.	-0.00947	0.43170	0.04305
	0.5248	0.0742	**0.0192**	0.4348	0.3482	0.3086	0.9655	0.2444	.	0.9319	**0.0397**	0.6992
16:0	0.01202	0.08452	-0.11431	-0.08404	0.05261	-0.38562	-0.08764	-0.08331	.	0.07046	-0.17337	0.11309
	0.9146	0.7646	0.3005	0.4472	0.6346	0.1402	0.7395	0.4540	.	0.5242	0.7289	0.3087
16:1	0.09637	0.15122	-0.17473	-0.06319	0.09973	-0.05292	-0.00312	-0.09836	.	0.01820	-0.22106	-0.03165
	0.3891	0.5906	0.1119	0.5680	0.3667	0.8457	0.9781	0.3763	.	0.8695	0.3107	0.7764
17:0	-0.03247	0.38565	0.17848	-0.02674	-0.09964	-0.06602	-0.01872	0.00768	.	0.06832	0.26851	0.06137
	0.7721	0.1557	0.1043	0.8092	0.3672	0.8081	0.8691	0.9451	.	0.5369	0.2154	0.5815
18:0	-0.03787	0.22598	0.07413	0.20504	0.13529	0.18367	0.11991	0.14105	.	0.03386	0.11317	0.19394
	0.7355	0.4180	0.5027	0.0613	0.2198	0.4959	0.2894	0.2034	.	0.7598	0.6072	0.0789
18:1n-9	0.09608	0.20309	-0.12235	0.14123	0.14496	0.28138	0.10531	0.04463	.	0.03494	-0.80412	0.11392
	0.3905	0.4679	0.2676	0.2000	0.1883	0.2911	0.3525	0.6887	.	0.7523	0.7027	0.3052
18:2n-6	0.06081	-0.09185	0.32760	0.05857	-0.06322	0.16476	0.02937	0.13232	.	0.02650	0.48411	-0.04773
	0.5873	0.7448	**0.0024**	0.5967	0.5678	0.5420	0.7959	0.2331	.	0.8109	**0.0192**	0.6683
20:0	-0.02212	0.23611	0.09921	0.03106	-0.05525	-0.12366	-0.12367	0.00220	.	-0.04521	0.41556	0.03010
	0.8436	0.3969	0.3693	0.7791	0.6176	0.6482	0.2744	0.9843	.	0.6830	**0.0486**	0.7871
18:3n-6	-0.11672	-0.30782	0.14479	0.15550	-0.00300	0.34191	-0.18083	0.08109	.	-0.07552	0.48650	-0.05833
	0.2963	0.2644	0.1888	0.1578	0.9784	0.1949	0.1085	0.4662	.	0.4948	**0.0186**	0.6004
20:1	0.14736	-0.04628	0.26856	0.18180	-0.06040	0.30391	-0.00980	0.16947	.	-0.03278	0.31071	0.02087
	0.1864	0.8699	**0.0135**	0.0979	0.5852	0.2525	0.9312	0.1256	.	0.7672	0.1490	0.8514
18:3n-3	0.22326	-0.00116	0.34634	0.08074	0.03044	0.27779	0.09391	0.19238	.	0.17680	0.55254	0.12085
	0.0438	0.9967	**0.0013**	0.4654	0.7834	0.2976	0.4073	0.0814	.	0.1077	**0.0063**	0.2765
20:2	-0.05744	-0.08467	0.02262	0.10897	-0.06281	-0.03312	-0.19532	0.02262	.	-0.08251	0.22866	-0.06404
	0.6082	0.7642	0.8382	0.3238	0.5703	0.9031	0.0825	0.8391	.	0.4556	0.2940	0.5651
20:3n-6	-0.22078	-0.48416	0.02549	0.19753	-0.11831	0.35206	-0.04995	0.16850	.	-0.11475	0.28811	-0.11703
	0.0462	0.0674	0.8179	0.0717	0.2838	0.1811	0.6599	0.1278	.	0.2986	0.1825	0.2921
22:1n-9	0.18257	0.17020	0.09801	-0.07855	-0.01807	-0.28399	-0.12293	-0.09600	.	0.01132	-0.24044	-0.00943
	0.1007	0.5442	0.3751	0.4776	0.8704	0.2864	0.2773	0.3879	.	0.9186	0.2691	0.9326
20:3n-3	0.04645	0.13198	0.25877	0.12983	-0.05093	0.48264	0.20352	0.27488	.	0.05110	0.28437	0.15540
	0.6786	0.6392	**0.0175**	0.2392	0.6455	0.0583	0.0702	**0.0119**	.	0.6444	0.1885	0.1607
20:4n-6	-0.14072	-0.20500	0.07682	-0.13611	-0.01520	-0.25915	-0.12811	-0.12895	.	-0.12031	-0.14999	-0.19020
	0.2073	0.4636	0.4873	0.2170	0.1677	0.3324	0.2574	0.2453	.	0.2757	0.4945	0.0850
20:5n-3	-0.12198	0.05208	0.09511	-0.11543	-0.18197	-0.08691	-0.00440	-0.01009	.	-0.04786	-0.26664	-0.12253
	0.2750	0.8538	0.3895	0.2958	0.0976	0.7489	0.9691	0.9278	.	0.6655	0.2188	0.2698
24:1	-0.14165	-0.09124	0.07068	-0.08282	-0.15083	-0.01285	-0.04945	0.00090	.	0.13428	0.18330	-0.15873
	0.2043	0.7464	0.5229	0.4539	0.1708	0.9623	0.6632	0.9936	.	0.2233	0.5908	0.1518
22:6n-3	-0.20617	-0.19781	0.00479	-0.17170	-0.20842	-0.22583	-0.09811	-0.09625	.	-0.13298	-0.07157	-0.21322
	0.0631	0.4798	0.9655	0.1184	0.0571	0.4003	0.3866	0.3867	.	0.2279	0.7455	0.0529
SAT	0.01147	0.09645	-0.09748	-0.06722	0.06280	-0.35533	-0.07465	-0.06765	.	0.07455	-0.16057	0.12743
	0.9186	0.7324	0.3777	0.5435	0.5740	0.1768	0.5105	0.5434	.	0.5003	0.4642	0.2510
MUFA	0.10518	0.20098	-0.13538	0.12675	0.14916	0.25579	0.09897	0.03091	.	0.03491	-0.10130	0.10335
	0.3470	0.4726	0.2195	0.2506	0.1757	0.3390	0.3838	0.7815	.	0.7526	0.6456	0.3525
PUFA	-0.10523	-0.19622	0.18112	-0.08115	-0.17467	-0.03353	-0.05131	0.00876	.	-0.07427	0.15153	-0.16881
	0.3468	0.4834	0.0992	0.4631	0.1120	0.9019	0.6513	0.9374	.	0.5020	0.4901	0.1271
ω3	-0.20222	-0.19199	0.01189	-0.16993	-0.20865	-0.21804	-0.09414	-0.09158	.	-0.12883	-0.07527	-0.21002
	0.0685	0.4930	0.9145	0.1223	0.0568	0.4172	0.4062	0.4103	.	0.2428	0.7329	0.0567
ω6	0.05640	-0.09857	0.32481	0.06099	-0.06585	0.16153	0.02187	0.13109	.	0.02233	0.48499	-0.05092
	0.6148	0.7267	**0.0026**	0.5815	0.5518	0.5501	0.8473	0.2375	.	0.8402	**0.0190**	**0.6475**
ω3:ω6	-0.23958	-0.19913	-0.13734	-0.19404	-0.21226	-0.24617	-0.10914	-0.14739	.	-0.15811	-0.25113	-0.22891
	0.0302	0.4768	0.2128	0.0770	0.0526	0.3581	0.3352	0.1836	.	0.1509	0.2477	**0.0374**
n-value	82	15	84	84	84	16	80	83	.	84	23	83

APPENDIX 9

TROUT GENOME PROJECT: CORRELATIONS WITH VISCERAL ADIPOSE TISSUE GENE EXPRESSION

[Each table indicates the R^2-value (top number) and p-value (bottom number) for each correlation.]

Table 21: Correlations between Phenotypic Traits and Lipogenic Gene Expression in Visceral Adipose Tissue

	Lipogenic and Fatty Acid Transport Genes										
	gpat	*srebp1*	*acyl*	*acc*	*fas*	*scd1*	*fabp3*	*fabp4*	*lpl*	*cd36*	*me*
WBW	0.08585	.	0.05171	-0.20395	-0.08721	0.05360	0.08583	0.05235	0.13270	-0.17489	-0.09701
	0.4519	.	0.7158	0.0857	0.4477	0.7459	0.4520	0.6555	0.2347	0.1184	0.4769
GIT	0.09366	.	-0.14046	-0.22460	-0.18322	-0.04612	0.12437	-0.09845	0.11985	-0.20598	0.13276
	0.4117	.	0.3206	0.0579	0.1084	0.7804	0.2748	0.4007	0.2835	0.0651	0.3293
GSI	0.00461	.	-0.12196	-0.01598	-0.13186	0.17175	0.03814	0.17574	-0.06875	-0.19557	-0.12006
	0.9680	.	0.3939	0.8948	0.2498	0.2958	0.7403	0.1342	0.5420	0.0821	0.3781
SepMus	-0.00505	.	-0.02593	-0.06583	0.05905	-0.06577	0.02368	-0.00895	0.09975	0.05928	-0.04976
	0.9648	.	0.8552	0.5827	0.6076	0.6908	0.8359	0.9393	0.3726	0.5991	0.7157
Trim	-0.01530	.	0.11039	0.23410	0.06822	-0.02328	-0.19659	0.06219	-0.26074	0.15240	0.02510
	0.8936	.	0.4360	**0.0478**	0.5529	0.8881	0.0825	0.5961	0.0180	0.1744	0.8543
BF	0.00381	.	0.12991	-0.26004	-0.09735	0.02839	0.06893	-0.06086	0.05305	-0.05548	0.01180
	0.9734	.	0.3587	0.0274	0.3965	0.8638	0.5461	0.6040	0.6359	0.6228	0.9312
MusFat	-0.09231	.	0.03474	-0.10259	-0.16463	-0.00320	0.19035	-0.04413	0.03818	-0.11560	-0.06155
	0.4185	.	0.8069	0.3912	0.1498	0.9846	0.0929	0.7070	0.7334	0.3041	0.6522
n-value	79	0	52	72	78	39	79	75	82	81	56

Table 22: Correlations between Phenotypic Traits and β-Oxidation Gene Expression in Visceral Adipose Tissue

	β-oxidation Genes											
	magl	*cpt1a*	*cpt1b*	*cpt1c*	*cpt1d*	*cpt2*	*aco*	*acdh*	*acdhm*	*acdhvl*	*ehhadh*	*acat2*
WBW	0.04721	-0.07573	0.13281	-0.16518	-0.02148	0.29590	-0.03886	0.06359	-0.27178	-0.03084	-0.19325	0.17972
	0.6795	0.5686	0.2593	0.1381	0.8481	0.3043	0.7305	0.7385	0.5555	0.8043	0.0819	0.1084
GIT	-0.23827	0.11774	0.05668	-0.29671	0.11859	-0.22667	-0.15368	0.18277	-0.10116	-0.18992	-0.03341	0.22076
	0.0345	0.3745	0.6315	**0.0068**	0.2886	0.4358	0.1708	0.3337	0.8291	0.1237	0.7657	**0.0476**
GSI	0.00543	-0.17410	0.22245	-0.24933	-0.02463	-0.00638	-0.10391	0.15037	0.07642	-0.11978	-0.25646	0.15785
	0.9624	0.1872	0.0585	0.0248	0.8272	0.9827	0.3590	0.4362	0.8706	0.3381	**0.0208**	0.1620
SepMus	0.12376	-0.05067	0.03593	0.03536	-0.18295	0.12361	0.07206	0.16138	-0.29535	-0.01672	-0.11634	-0.02293
	0.2772	0.7031	0.7612	0.7525	0.0999	0.6737	0.5226	0.3942	0.5202	0.8932	0.2979	0.8390
Trim	0.10727	-0.04291	-0.09717	0.21502	0.11450	-0.05247	0.09740	-0.26296	0.85514	0.11443	0.14768	-0.22744
	0.3467	0.7470	0.4102	0.0524	0.3057	0.8586	0.3870	0.1603	0.0142	0.3565	0.1855	**0.0411**
BF	-0.09801	-0.06155	0.07428	-0.09734	0.14877	0.20215	-0.11865	0.14997	-0.44487	-0.03746	-0.19729	0.21602
	0.3901	0.6433	0.5294	0.3843	0.1822	0.4883	0.2914	0.4289	0.3172	0.7634	0.0756	0.0528
MusFat	-0.08647	-0.12083	0.11561	-0.07252	0.16074	0.33183	-0.12407	0.07398	-0.76305	-0.02617	-0.22568	0.21754
	0.4486	0.3620	0.3266	0.5173	0.1491	0.2464	0.2698	0.6976	0.0460	0.8335	**0.0415**	0.0511
n-value	79	59	74	82	82	14	81	30	7	67	82	81

Table 23: Correlations between Phenotypic Traits and Signaling Factor Gene Expression in Visceral Adipose Tissue

	Signaling Factors											
	erk	*akt2*	*redd1*	*mo25*	*mtor*	*raptor*	*rictor*	*pras40*	*ppar α*	*ppar β*	*ppar γ*	*rxr*
WBW	0.06853	0.14815	-0.16967	-0.04593	-0.14413	-0.24182	0.08664	0.17160	-0.55908	-0.03861	-0.15035	-0.01001
	0.5432	0.2046	0.1275	0.6839	0.1964	0.3852	0.4448	0.1232	0.1497	0.7306	0.2732	0.9293
GIT	-0.01409	0.04590	-0.42353	-0.05378	-0.23797	0.19653	-0.03264	-0.11434	0.27379	-0.28857	0.16047	-0.17398
	0.9006	0.6958	**<.0001**	0.6335	**0.0313**	0.4827	0.7738	0.3064	0.5117	**0.0086**	0.2419	0.1203
GSI	-0.06853	0.25467	-0.09683	-0.11869	-0.25827	-0.00837	-0.13455	-0.15481	0.36592	-0.16440	-0.07565	-0.08310
	0.5458	**0.0285**	0.3898	0.2944	**0.0199**	0.9764	0.2371	0.1676	0.3727	0.1425	0.5866	0.4637
SepMus	-0.00077	0.06020	-0.00797	-0.08544	0.05511	-0.00569	0.04864	0.16724	-0.75757	0.16916	-0.12810	0.12558
	0.9946	0.6079	0.9434	0.4482	0.6229	0.9840	0.6683	0.1331	**0.0295**	0.1287	0.3513	0.2640
Trim	-0.10018	-0.10623	0.33733	0.10027	0.15552	-0.19344	0.02357	-0.06071	0.43039	0.09411	0.07813	-0.00399
	0.3735	0.3644	**0.0019**	0.3731	0.1630	0.4897	0.8356	0.5880	0.2871	0.4004	0.5707	0.9718
BF	-0.04408	0.19147	-0.20160	-0.08144	-0.15415	-0.44690	0.00033	0.12012	-0.29168	-0.04701	0.01858	-0.03753
	0.6960	0.0998	0.0693	0.4698	0.1668	0.0949	0.9977	0.2824	0.4833	0.6749	0.8929	0.7394
MusFat	0.01482	0.18561	-0.19408	-0.02913	-0.12622	-0.20261	-0.07383	0.05240	-0.07139	-0.10654	-0.07338	-0.15582
	0.8955	0.1109	0.0806	0.7963	0.2585	0.4689	0.5151	0.6401	0.8666	0.3408	0.5944	0.1648
n-value	81	75	82	81	82	15	80	82	8	82	55	81

Table 24: Correlations between Fatty Acid Composition and Lipogenic Gene Expression in Visceral Adipose Tissue

	Lipogenic and Fatty Acid Gene Expression										
	gpat	*srebp1*	*acyl*	*acc*	*fas*	*scd1*	*fabp3*	*fabp4*	*lpl*	*cd36*	*me*
12:0	0.24742	.	0.09651	0.33581	0.16678	0.10424	0.06079	0.01935	0.06959	0.43881	0.15132
	0.0279	.	0.4961	0.0039	0.1445	0.5277	0.5946	0.8691	0.5344	**<0.0001**	0.2656
14:0	0.01321	.	-0.16623	0.07380	-0.10099	-0.21285	-0.16054	-0.14825	-0.12862	0.10891	0.12282
	0.9080	.	0.2389	0.5379	0.3790	0.1933	0.1576	0.2043	0.2495	0.3331	0.3672
14:1	0.15301	.	-0.02168	-0.04166	0.09785	0.09147	0.01247	-0.19505	0.05723	0.20817	0.10638
	0.1782	.	0.8788	0.7283	0.3941	0.5797	0.9132	0.0936	0.6095	0.0622	0.4352
15:0	-0.00599	.	0.01821	0.12129	0.01005	0.09796	-0.31371	-0.11007	-0.18876	0.15403	0.04481
	0.9582	.	0.8980	0.3101	0.9304	0.5530	**0.0049**	0.3472	0.0894	0.1698	0.7430
16:0	-0.00687	.	-0.18807	0.06586	-0.14230	-0.06864	-0.06300	-0.11012	-0.06888	0.04832	0.10461
	0.9521	.	0.1818	0.5826	0.2139	0.6780	0.5812	0.3470	0.5386	0.6684	0.4429
16:1	-0.07857	.	0.00326	0.05622	-0.01031	0.15327	0.26293	-0.13983	0.06962	0.01865	0.06686
	0.4913	.	0.9817	0.6390	0.9286	0.3516	0.0192	0.2315	0.5342	0.8687	0.6244
17:0	0.21399	.	-0.08149	0.06251	0.05804	-0.13976	-0.31570	0.07862	-0.06888	0.07368	0.04636
	0.0583	.	0.5658	0.6019	0.6137	0.3961	**0.0046**	0.5026	0.5387	0.5133	0.7344
18:0	-0.03655	.	-0.08647	0.03372	-0.08993	0.02608	-0.06654	0.25637	-0.11722	-0.09543	0.05282
	0.7491	.	0.5422	0.7786	0.4336	0.8748	0.5601	0.0264	0.2943	0.3967	0.6990
18:1n-9	-0.02484	.	0.08629	-0.12338	0.02482	0.12333	0.18150	0.21030	0.04883	-0.24007	-0.05753
	0.8280	.	0.5430	0.3018	0.8292	0.4545	0.1094	0.0701	0.6631	**0.0309**	0.6737
18:2n-6	-0.01090	.	-0.06109	0.15228	0.02372	-0.18875	-0.11877	-0.13255	0.01754	0.21209	-0.00811
	0.9240	.	0.6670	0.2016	0.8367	0.2498	0.2972	0.2569	0.8757	0.0573	0.9527
20:0	0.14606	.	-0.05068	-0.03413	-0.04465	-0.12479	-0.10476	0.06716	-0.03622	0.19853	-0.03773
	0.1990	.	0.7213	0.7760	0.6979	0.4491	0.3582	0.5670	0.7467	0.0756	0.7825
18:3n-6	-0.09619	.	0.06044	0.10633	0.11715	-0.10037	-0.06372	-0.04765	-0.01625	0.02710	0.04866
	0.3991	.	0.6704	0.3740	0.3070	0.5432	0.5769	0.6848	0.8848	0.8102	0.7217
20:1	0.03463	.	-0.05887	0.03929	-0.06717	-0.23949	-0.10275	0.15471	-0.10187	0.15398	-0.00483
	0.7619	.	0.6785	0.7432	0.5590	0.1420	0.3675	0.1851	0.3625	0.1699	0.9718
18:3n-3	-0.05423	.	-0.12282	0.00918	-0.08129	-0.14804	-0.13953	-0.08055	-0.01496	0.14656	0.00264
	0.6350	.	0.3857	0.9390	0.4793	0.3684	0.2200	0.4921	0.8939	0.1917	0.9846
20:2	0.12961	.	0.02541	0.11911	-0.00488	-0.10821	-0.24903	0.14519	-0.22398	0.01502	0.13997
	0.2549	.	0.8581	0.3190	0.9662	0.5120	**0.0269**	0.2139	**0.0431**	0.8941	0.3035
20:3n-6	0.14954	.	0.06107	0.01217	0.05116	-0.01936	-0.10786	-0.04147	-0.09704	0.11235	0.05745
	0.1884	.	0.6671	0.9192	0.6565	0.9069	0.3441	0.7239	0.3858	0.3180	0.6741
22:1n-9	-0.05807	.	-0.02777	0.09286	0.01601	0.03349	0.14270	0.01038	-0.00794	-0.01280	0.04740
	0.6112	.	0.8450	0.4378	0.8894	0.8396	0.2096	0.9295	0.9435	0.9097	0.7286
20:3n-3	-0.06164	.	-0.14712	0.01927	-0.10285	-0.20830	-0.02582	-0.17112	0.05235	0.05317	-0.07807
	0.5894	.	0.2980	0.8724	0.3702	0.2032	0.8213	0.1421	0.6404	0.6373	0.5674
20:4n-6	0.08052	.	0.05204	-0.01869	0.10444	0.08656	-0.04161	-0.05904	0.00039	0.04819	-0.09141
	0.4805	.	0.7141	0.8761	0.3628	0.6003	0.7158	0.6148	0.9972	0.6692	0.5028
20:5n-3	0.03745	.	0.05663	0.06256	0.08107	-0.00033	-0.12151	-0.25019	-0.01146	0.11035	-0.18307
	0.7432	.	0.6901	0.6016	0.4804	0.9984	0.2861	0.0304	0.9186	0.3267	0.1769
24:1	0.05099	.	0.00758	0.10296	-0.02358	-0.10124	-0.18814	0.06262	-0.13340	0.14562	-0.00235
	0.6554	.	0.9574	0.3894	0.8377	0.5397	0.0968	0.5936	0.2322	0.1946	0.9863
22:6n-3	0.06674	.	0.11172	-0.03364	0.08242	0.00862	-0.12992	-0.10081	-0.02532	0.13363	-0.03587
	0.5589	.	0.4304	0.7790	0.4731	0.9585	0.2538	0.3895	0.8213	0.2343	0.7930
SAT	-0.00369	.	-0.18732	0.07372	-0.14106	-0.06623	-0.06785	-0.09131	-0.07546	0.05135	0.10973
	0.9743	.	0.1836	0.5383	0.2180	0.6887	0.5524	0.4359	0.5004	0.6489	0.4208
MUFA	-0.03350	.	0.08141	-0.11036	0.02153	0.13390	0.20465	0.18689	0.05441	-0.22569	-0.04233
	0.7694	.	0.5661	0.3561	0.8515	0.4164	0.0704	0.1084	0.6273	**0.0428**	0.7567
PUFA	0.03717	.	0.03714	0.06695	0.06333	-0.09235	-0.15428	-0.14081	-0.00953	0.20379	-0.02845
	0.7450	.	0.7938	0.5763	0.5817	0.5760	0.1746	0.2282	0.9323	0.0680	0.8351
ω3	0.06495	.	0.10816	-0.02891	0.08181	0.00668	-0.13182	-0.11003	-0.02491	0.13486	-0.04380
	0.5696	.	0.4453	0.8095	0.4764	0.9678	0.2469	0.3474	0.8242	0.2300	0.7486
ω6	-0.00520	.	-0.05942	0.15411	0.02420	-0.18947	-0.12646	-0.12771	0.00962	0.21043	-0.00381
	0.9637	.	0.6756	0.1962	0.8334	0.2480	0.2668	0.2749	0.9316	0.0593	0.9778
ω3:ω6	0.08497	.	0.13567	-0.12055	0.08485	0.12617	-0.04792	-0.08316	0.00619	0.04190	-0.05513
	0.4565	.	0.3376	0.3131	0.4601	0.4440	0.6749	0.4781	0.9560	0.7104	0.6866
n-value	79	0	52	72	78	39	79	75	82	81	56

Table 25: Correlations between Fatty Acid Composition and β-Oxidation Gene Expression in Visceral Adipose Tissue

	β-oxidation Genes											
	magl	*cpt1a*	*cpt1b*	*cpt1c*	*cpt1d*	*cpt2*	*aco*	*acdh*	*acdhm*	*acdhvl*	*ehhadh*	*acat2*
12:0	0.19949	0.25836	0.28720	0.35531	0.00104	0.44505	0.19963	0.00545	0.20260	0.11384	0.28247	0.07441
	0.0780	**0.0482**	**0.0131**	**0.0011**	0.9926	0.1108	0.0740	0.9772	0.6631	0.3590	**0.0101**	0.5091
14:0	0.07019	0.24643	0.05781	0.24068	0.00712	-0.24972	0.03016	-0.38353	-0.61894	0.21080	0.09507	0.05105
	0.5388	0.0599	0.6246	**0.0294**	0.9494	0.3892	0.7892	**0.0364**	0.1384	0.0868	0.3955	0.6509
14:1	-0.09608	-0.00007	0.07926	0.09310	0.11127	0.49078	0.16441	0.25496	-0.57682	0.10177	0.02052	0.12995
	0.3996	0.9996	0.5021	0.4055	0.3196	0.0748	0.1425	0.1739	0.1752	0.4125	0.8548	0.2476
15:0	0.03066	0.08532	0.04383	0.15334	0.24204	-0.07991	0.18478	-0.27887	0.53173	0.15242	0.07366	-0.12429
	0.7885	0.5205	0.7108	0.1690	**0.0285**	0.7860	0.0987	0.1356	0.2193	0.2182	0.5107	0.2689
16:0	0.08503	0.34191	-0.03309	0.16508	0.06888	-0.15700	-0.06430	-0.30661	-0.18775	0.17971	0.13403	0.05592
	0.4562	**0.0080**	0.7796	0.1383	0.5386	0.5919	0.5685	0.0994	0.6869	0.1456	0.2299	0.6200
16:1	-0.09829	-0.11944	0.10016	-0.01413	0.04800	0.39854	-0.02979	0.18527	-0.62069	0.03717	-0.07193	0.20300
	0.3888	0.3676	0.3958	0.8997	0.6685	0.1581	0.7918	0.3270	0.1369	0.7652	0.5207	0.0691
17:0	0.21134	0.23779	0.11411	0.13364	-0.01387	-0.24464	0.15475	-0.45938	0.89307	0.06990	0.03405	-0.09357
	0.0615	0.0697	0.3330	0.2313	0.9016	0.3993	0.1678	**0.0107**	**0.0068**	0.5740	0.7614	0.4060
18:0	0.10248	0.16862	-0.11910	-0.04107	0.07303	0.16604	0.08476	0.06847	0.28224	0.10788	0.11133	0.01575
	0.3688	0.2017	0.3122	0.7141	0.5144	0.5705	0.4519	0.7192	0.5397	0.3849	0.3194	0.8890
18:1n-9	-0.15204	-0.20244	-0.11371	-0.28644	-0.03061	0.35578	-0.03851	0.47777	-0.63486	-0.15128	-0.22677	0.06408
	0.1810	0.1241	0.3347	**0.0091**	0.7849	0.2119	0.7329	**0.0076**	0.1256	0.2217	**0.0405**	0.5698
18:2n-6	0.12383	0.10074	0.20314	0.13779	-0.07573	-0.23120	0.11787	-0.34407	0.36457	0.14009	0.17046	-0.02945
	0.2769	0.4478	0.0826	0.2170	0.4989	0.4265	0.2946	0.0626	0.4214	0.2582	0.1257	0.7941
20:0	0.04388	0.18020	0.18213	-0.03752	-0.00212	-0.35577	0.09958	0.25106	0.41908	-0.05022	0.13259	0.05031
	0.7010	0.1720	0.1204	0.7378	0.9849	0.2119	0.3764	0.1808	0.3493	0.6865	0.2350	0.6556
18:3n-6	-0.00190	-0.09383	0.00495	0.07135	-0.17287	-0.17443	0.06545	-0.29191	-0.11432	0.05658	0.11237	-0.15311
	0.9867	0.4797	0.9666	0.5241	0.1204	0.5509	0.5615	0.1175	0.8072	0.6493	0.3149	0.1724
20:1	0.00039	0.16037	0.15485	0.05238	-0.04251	-0.33483	0.16288	0.25992	0.34355	0.19824	0.15024	0.01314
	0.9973	0.2250	0.1877	0.6403	0.7045	0.2419	0.1463	0.1654	0.4506	0.1078	0.1779	0.9073
18:3n-3	0.00694	0.00310	0.09948	0.05584	-0.00561	-0.03466	0.11779	-0.22959	0.44294	0.06924	0.17577	-0.00692
	0.9516	0.9814	0.3991	0.6183	0.9601	0.9064	0.2950	0.2223	0.3196	0.5777	0.1142	0.9511
20:2	0.02671	0.37195	-0.12495	0.04203	-0.05698	-0.35939	0.18843	-0.03225	0.42632	0.25227	0.23931	-0.24606
	0.8153	**0.0037**	0.2888	0.7077	0.6111	0.2069	0.0921	0.8657	0.3402	**0.0394**	0.0304	**0.0268**
20:3n-6	0.03796	0.13359	-0.05814	0.15137	-0.08120	-0.12856	0.12720	0.00144	0.52057	0.09542	0.16308	-0.22965
	0.7398	0.3131	0.6227	0.1746	0.4683	0.6614	0.2578	0.9940	0.2310	0.4424	0.1432	**0.0392**
22:1n-9	0.09498	-0.08098	0.11335	-0.10345	0.01468	0.20834	0.07006	0.10102	0.27799	0.08391	0.00780	0.13787
	0.4050	0.5421	0.3363	0.3550	0.8959	0.4748	0.5343	0.5953	0.5461	0.4996	0.9446	0.2197
20:3n-3	-0.06788	0.02445	0.05221	0.02086	0.05324	-0.13471	0.07619	0.10528	0.73400	0.03182	0.09834	0.03467
	0.5523	0.8542	0.6586	0.8524	0.6348	0.6461	0.4990	0.5798	0.0604	0.7982	0.3794	0.7587
20:4n-6	0.06489	-0.11424	0.01734	0.08046	-0.00636	-0.17867	0.01138	-0.19715	0.33537	-0.12180	0.06940	-0.12927
	0.5700	0.3889	0.8834	0.4724	0.9548	0.5411	0.9197	0.2964	0.4621	0.3261	0.5355	0.2501
20:5n-3	0.06773	-0.13547	0.08944	0.18001	0.05096	-0.18958	0.04487	-0.25004	0.26810	0.03070	0.04662	-0.03598
	0.5531	0.3063	0.4485	0.1056	0.6493	0.5162	0.6908	0.1827	0.5610	0.8052	0.6775	0.7498
24:1	0.00517	-0.00402	-0.02467	0.08065	0.07299	-0.42026	0.14574	-0.00776	0.61044	0.05491	0.09244	-0.14530
	0.9639	0.9759	0.8347	0.4713	0.5146	0.1346	0.1942	0.9676	0.1454	0.6590	0.4088	0.1956
22:6n-3	0.05423	-0.09333	-0.01779	0.16094	0.04111	-0.29031	-0.00399	-0.20624	0.42418	-0.09542	0.06385	-0.17491
	0.6350	0.4820	0.8804	0.1486	0.7138	0.3140	0.9718	0.2742	0.3429	0.4424	0.5688	0.1183
SAT	0.09361	0.35347	-0.03239	0.16797	0.07090	-0.11336	-0.05101	-0.30069	-0.17355	0.18641	0.14325	0.05699
	0.4119	**0.0060**	0.7841	0.1314	0.5268	0.6996	0.6511	0.1064	0.7098	0.1309	0.1992	0.6133
MUFA	-0.15833	-0.20345	-0.08959	-0.27534	-0.02356	0.39930	-0.03822	0.47491	-0.65184	-0.13944	-0.22442	0.08802
	0.1634	0.1222	0.4478	**0.0123**	0.8336	0.1572	0.7348	**0.0080**	0.1126	0.2604	**0.0427**	0.4346
PUFA	0.10504	-0.00080	0.10843	0.17952	-0.01933	-0.32201	0.07051	-0.35577	0.55043	0.03074	0.14243	-0.12553
	0.3569	0.9952	0.3578	0.1066	0.8632	0.2615	0.5317	0.0537	0.2004	0.8050	0.2018	0.2642
ω3	0.05527	-0.09600	-0.01101	0.16342	0.04179	-0.28633	-0.00014	-0.21180	0.42443	-0.08827	0.06535	-0.16883
	0.6286	0.4695	0.9258	0.1424	0.7093	0.3210	0.9990	0.2612	0.3426	0.4775	0.5597	0.1319
ω6	0.12366	0.11189	0.19549	0.13854	-0.07705	-0.24846	0.12317	-0.34449	0.38124	0.14573	0.17720	-0.03902
	0.2776	0.3988	0.0951	0.2145	0.4915	0.3917	0.2733	0.0623	0.3988	0.2393	0.1113	0.7295
ω3:ω6	-0.04130	-0.14605	-0.08814	0.08685	0.08369	-0.18466	-0.06659	-0.05548	0.35203	-0.17528	-0.02346	-0.14849
	0.7178	0.2697	0.4552	0.4378	0.4548	0.5274	0.5548	0.7709	0.4387	0.1560	0.8343	0.1858
n-value	79	59	74	82	82	14	81	30	7	67	82	81

Table 26: Correlations between Fatty Acid Composition and Signaling Factor Gene Expression in Visceral Adipose Tissue

	Signaling Factors											
	erk	*akt2*	*redd1*	*mo25*	*mtor*	*raptor*	*rictor*	*pras40*	*ppar α*	*ppar β*	*ppar γ*	*rxr*
12:0	0.12621	-0.17919	0.50981	0.16786	0.31003	0.17868	0.29714	0.30910	0.07585	0.30151	0.07512	0.24950
	0.2616	0.1240	**<0.0001**	0.1341	**0.0046**	0.5240	**0.0074**	**0.0047**	0.8583	0.0059	0.5857	0.0247
14:0	-0.08237	-0.01161	0.04136	0.11720	0.12811	-0.19604	0.17347	0.23579	-0.28478	-0.00848	0.03428	0.04628
	0.4648	0.9212	0.7121	0.2974	0.2514	0.4838	0.1239	**0.0330**	0.4942	0.9397	0.8038	0.6816
14:1	-0.16757	0.02751	-0.06949	0.12845	0.08209	-0.15700	0.18014	0.29137	-0.02987	0.00081	0.00618	-0.03790
	0.1348	0.8147	0.5350	0.2531	0.4635	0.5763	0.1098	**0.0079**	0.9440	0.9943	0.9643	0.7369
15:0	-0.24016	-0.16095	0.19482	0.05610	0.15306	0.26126	0.04420	-0.00928	0.09459	0.09370	0.17976	0.09701
	0.0308	0.1678	0.0794	0.6189	0.1698	0.3469	0.6971	0.9341	0.8237	0.4024	0.1891	0.3889
16:0	-0.10191	0.00456	-0.13291	0.02804	0.04135	0.14589	0.13069	0.15049	0.04936	-0.08422	0.21451	0.06518
	0.3653	0.9690	0.2339	0.8037	0.7123	0.6039	0.2479	0.1772	0.9076	0.4519	0.1158	0.5632
16:1	0.08239	0.20221	-0.18807	-0.08096	-0.01846	-0.27349	-0.13137	-0.08128	-0.26924	-0.09987	-0.12401	-0.07964
	0.4647	0.0819	0.0906	0.4724	0.8693	0.3240	0.2454	0.4679	0.5190	0.3720	0.3670	0.4797
17:0	-0.09190	-0.21905	0.24799	0.10675	0.07035	0.11850	0.30408	0.16710	-0.32777	0.15392	0.07283	0.13542
	0.4145	0.0590	**0.0247**	0.3429	0.5299	0.6740	**0.0061**	0.1335	0.4280	0.1674	0.5972	0.2280
18:0	0.07958	0.05810	0.08465	0.02251	-0.01520	0.61030	0.11375	0.12111	0.60501	-0.03389	0.19619	0.00694
	0.4801	0.6205	0.4496	0.8419	0.8922	0.0157	0.3151	0.2784	0.1120	0.7625	0.1511	0.9510
18:1n-9	0.15485	0.14161	-0.21448	-0.17210	-0.20262	0.03810	-0.09280	-0.11800	-0.00996	-0.05099	-0.02883	-0.10922
	0.1675	0.2256	0.0530	0.1245	0.0679	0.8928	0.4129	0.2911	0.9813	0.6491	0.8345	0.3317
18:2n-6	-0.08761	-0.08468	0.34322	0.13333	0.16692	-0.19065	0.07330	0.13254	-0.52988	0.07142	-0.06724	0.02324
	0.4367	0.4701	**0.0016**	0.2354	0.1339	0.4961	0.5182	0.2352	0.1768	0.5237	0.6257	0.8369
20:0	-0.12695	-0.00783	0.05578	0.06336	-0.00329	0.00959	-0.02781	-0.03208	-0.41810	-0.03763	0.28034	-0.00825
	0.2588	0.9468	0.6187	0.5741	0.9766	0.9729	0.8065	0.7748	0.3026	0.7372	**0.0382**	0.9417
18:3n-6	0.02991	0.01653	0.13442	0.07552	0.16482	-0.12124	-0.03595	-0.12510	-0.17959	0.05141	-0.10996	0.02021
	0.7910	0.8880	0.2286	0.5028	0.1389	0.6669	0.7516	0.2628	0.6704	0.6464	0.4242	0.8579
20:1	-0.06592	0.10769	0.20265	0.21826	0.08640	-0.16455	0.03460	0.12286	-0.40466	-0.06200	0.24736	-0.10869
	0.5588	0.3578	0.0679	**0.0503**	0.4402	0.5578	0.7606	0.2715	0.3200	0.5800	0.0686	0.3341
18:3n-3	-0.04381	-0.09333	0.28048	0.14500	0.09165	-0.21755	0.02662	0.10561	-0.22653	0.01647	-0.07718	-0.04957
	0.6978	0.4258	**0.0107**	0.1965	0.4128	0.4360	0.8147	0.3450	0.5896	0.8833	0.5754	0.6603
20:2	-0.13618	-0.04243	0.05301	0.11892	0.09810	-0.01788	0.06552	0.02465	0.19754	-0.01612	0.48077	0.01842
	0.2254	0.7178	0.6362	0.2903	0.3806	0.9496	0.5637	0.8260	0.6391	0.8857	**0.0002**	0.8703
20:3n-6	-0.18697	-0.05320	-0.00681	0.30087	0.13753	-0.41976	0.12635	0.09076	0.03243	0.04863	0.19259	-0.01955
	0.0947	0.6503	0.9516	**0.0063**	0.2179	0.1193	0.2641	0.4174	0.9392	0.6644	0.1589	0.8625
22:1n-9	-0.09481	0.17345	0.02675	-0.10350	-0.01303	0.33605	0.10718	0.06156	0.15915	-0.08247	-0.03568	-0.11017
	0.3999	0.1367	0.8115	0.3578	0.9075	0.2207	0.3440	0.5827	0.7066	0.4614	0.7959	0.3275
20:3n-3	-0.19448	-0.12041	0.01811	0.06917	0.03405	-0.09309	-0.00204	0.14450	-0.42331	-0.01143	0.15742	-0.00492
	0.0819	0.3035	0.8717	0.5395	0.7614	0.7414	0.9856	0.1952	0.2960	0.9188	0.2511	0.9652
20:4n-6	0.01883	-0.20429	0.27567	0.10065	0.06667	0.20444	-0.04049	-0.10056	0.48967	0.04940	-0.13419	0.07493
	0.8675	0.0787	**0.0122**	0.3713	0.5518	0.4649	0.7214	0.3687	0.2181	0.6594	0.3287	0.5061
20:5n-3	-0.05433	-0.27100	0.15875	0.07630	0.11823	0.10919	-0.04490	-0.02138	0.34168	0.10970	-0.12499	0.12313
	0.6300	**0.0187**	0.1543	0.4984	0.2901	0.6985	0.6925	0.8488	0.4075	0.3266	0.3632	0.2735
24:1	-0.15896	-0.06959	0.20734	0.02483	0.14940	-0.00863	-0.07214	-0.02219	0.04927	0.07895	0.23741	0.08792
	0.1564	0.5530	0.0616	0.8259	0.1804	0.9756	0.5249	0.8431	0.9078	0.4808	0.0809	0.4351
22:6n-3	-0.09066	-0.20908	0.16428	0.12686	0.11847	-0.00200	-0.03419	-0.08901	0.29654	0.11571	-0.10250	0.10352
	0.4209	0.0718	0.1403	0.2591	0.2891	0.9944	0.7634	0.4265	0.4757	0.3006	0.4565	0.3577
SAT	-0.09177	0.00429	-0.11007	0.03450	0.04786	0.20849	0.14265	0.16362	0.08018	-0.07684	0.22087	0.06879
	0.4152	0.9708	0.3249	0.7598	0.6694	0.4559	0.2068	0.1419	0.8503	0.4926	0.1051	0.5417
MUFA	0.15817	0.16402	-0.22702	-0.17236	-0.19516	-0.00403	-0.10553	-0.12187	-0.04855	-0.06260	-0.04177	-0.11629
	0.1584	0.1597	0.0403	0.1239	0.0789	0.9886	0.3515	0.2754	0.9091	0.5763	0.7621	0.3012
PUFA	-0.10803	-0.18276	0.30057	0.15663	0.17094	-0.09042	0.02127	0.02467	-0.03207	0.11141	-0.09830	0.07720
	0.3371	0.1166	**0.0061**	0.1626	0.1247	0.7486	0.8514	0.8259	0.9399	0.3190	0.4752	0.4934
ω3	-0.08984	-0.21488	0.16810	0.12666	0.12015	0.00221	-0.03461	-0.08476	0.29947	0.11620	-0.10502	0.10451
	0.4251	0.0641	0.1311	0.2598	0.2823	0.9938	0.7605	0.4490	0.4712	0.2985	0.4454	0.3532
ω6	-0.09164	-0.08671	0.34235	0.13738	0.16901	-0.18913	0.07472	0.13123	-0.52143	0.07051	-0.05006	0.02397
	0.4158	0.4595	**0.0016**	0.2213	0.1290	0.4996	0.5101	0.2399	0.1851	0.5290	0.7166	0.8318
ω3:ω6	-0.03898	-0.20261	-0.01079	0.09851	0.04620	0.00461	-0.08027	-0.15655	0.47355	0.06930	-0.08581	0.08344
	0.7297	0.0813	0.9233	0.3816	0.6802	0.9870	0.4791	0.1602	0.2359	0.5361	0.5333	0.4590
n-value	81	75	82	81	82	15	80	82	8	82	55	81

APPENDIX 10

EXAMPLE SAS CODES

Feeding Rate Study --- Ration Effects --- Example SAS Code

```
options formdlim = '-';

options pageno=1 pagesize=60;
Title 'Meg ration' ;

data GeXP;
input  sample $ month $ ID lot family $ ration $  ACAT2     ACC           ACDHM   ACDHVL  ACO
       ACYL    AKT2    CPT1a   CPT1b   CPT1c   CPT1d   EHHADH  ERK12   FABP3   FABP4   FAS     FATCD36
       GPAT    LPL     MAGL    MalicEnzyme     MCPT1a  MO25    mTOR    PPARalpha       PPARbeta
       PPARgamma       PRAS40  Raptor  REDD1   Rictor  RxR     SCD1    SREBP1
;
datalines;

;
proc glm data = GeXP;
class month ploidy ;
model ACAT2     ACC             ACDHM   ACDHVL  ACO     ACYL    AKT2    CPT1a   CPT1b   CPT1c   CPT1d
       EHHADH  ERK12   FABP3   FABP4   FAS     FATCD36 GPAT    LPL     MAGL    MalicEnzyme     MCPT1a
       MO25    mTOR    PPARalpha       PPARbeta        PPARgamma       PRAS40  Raptor  REDD1   Rictor
       RxR     SCD1 SREBP1      = month ration month*ration;
lsmeans month*ration /stderr pdiff;
run;
```

Feeding Rate Study --- Ploidy Effects --- Example SAS Code

```
options formdlim = '-';

options pageno=1 pagesize=60;
Title 'Meg ploidy' ;

data GeXP;
input  sample $ month $ ID lot family $ ploidy $  ACAT2     ACC           ACDHM   ACDHVL  ACO
       ACYL    AKT2    CPT1a   CPT1b   CPT1c   CPT1d   EHHADH  ERK12   FABP3   FABP4   FAS     FATCD36
       GPAT    LPL     MAGL    MalicEnzyme     MCPT1a  MO25    mTOR    PPARalpha       PPARbeta
       PPARgamma       PRAS40  Raptor  REDD1   Rictor  RxR     SCD1    SREBP1
;
datalines;

;
proc glm data = GeXP;
class month ploidy ;
model ACAT2     ACC             ACDHM   ACDHVL  ACO     ACYL    AKT2    CPT1a   CPT1b   CPT1c   CPT1d
       EHHADH  ERK12   FABP3   FABP4   FAS     FATCD36 GPAT    LPL     MAGL    MalicEnzyme     MCPT1a
       MO25    mTOR    PPARalpha       PPARbeta        PPARgamma       PRAS40  Raptor  REDD1   Rictor
       RxR     SCD1 SREBP1      = month ploidy month*ploidy;
lsmeans month*ploidy /stderr pdiff;
run;
```

2N/3N Maturation Study --- Example SAS Code

```
option LS=80 pagesize=60 nonumber;
title 'ANOVA_2n3n_ALL TISSUES';
data genes;
input tissue $ age ploidy $ family $ acat2 acc acdh acdhm acdhvl aco acyl akt2 cpt1a cpt1b cpt1c
cpt1d ehhadh erk fabp3 fabp4 fas cd36 gpat lpl magl malenz cpt2 mo25 mtor ppara pparb pparg
pras40 raptor redd1 rictor rxr scd1 srebp1;
datalines;

;
proc mixed data=genes method=reml;
class tissue age ploidy family;
model srebp1 = tissue age ploidy tissue*ploidy tissue*age tissue*age*ploidy ;
random family;
lsmeans tissue age ploidy tissue*ploidy tissue*age tissue*age*ploidy ;
run;
```

Male verses Female Study --- Example SAS Code

```
options ls=80;
data MegMvFFA;
input gender $ family $ c120 c130 c140 c141 c150 c160 c161 c170 c171 c180 c181n9 c182n6 c200
c183n6 c201 c183n3 c210 c202 c220 c203n6 c221n9 c203n3 c204n6 c222 c205n3 c241 c226n3;
datalines;

;
proc glm data=MegMvFFA ;
class gender family;
model c120 c130 c140 c141 c150 c160 c161 c170 c171 c180 c181n9 c182n6 c200 c183n6 c201 c183n3
c210 c202 c220 c203n6 c221n9 c203n3 c204n6 c222 c205n3 c241 c226n3=gender;
lsmeans gender /stderr pdiff;
run;
```

Trout Genome Study --- One-Way ANOVA --- Example SAS Code

```
options ls=80;
data MegTGFA;
input group $ family $ c120 c130 c140 c141 c150 c160 c161 c170 c171 c180 c181n9 c182n6 c200
c183n6 c201 c183n3 c210 c202 c220 c203n6 c221n9 c203n3 c204n6 c222 c205n3 c241 c226n3;
datalines;

;
proc glm data=MegTGFA ;
class group family;
model c120 c130 c140 c141 c150 c160 c161 c170 c171 c180 c181n9 c182n6 c200 c183n6 c201 c183n3
c210 c202 c220 c203n6 c221n9 c203n3 c204n6 c222 c205n3 c241 c226n3=group;
lsmeans group /stderr pdiff;
run;
```

Trout Genome Project --- Correlations --- Example SAS Code

```
options ls=80;
data MegsFAandliver;
input Group $  C12 C14 C141 C15 C16 C161 C17 C18 C181n9 C182n6 C20 C183n6 C201 C183n3 C202 C203n6
C221n9 C203n3 C204n6 C205n3 C241 C226n3 SFA MUFA PUFA W3 W6 W3toW6  ACAT2   ACC     ACDH    ACDHM
       ACDHVL  ACO     ACYL    AKT2    CPT1a   CPT1b   CPT1c   CPT1d   EHHADH  ERK12   FABP3   FABP4
       FAS     CD36    GPAT    LPL     MAGL    MalEnz  CPT2    MO25    mTOR    PPARa   PPARb   PPARg
       PRAS40  Raptor  REDD1   Rictor  RxR     SCD1    SREBP1
;
cards;

;
/*Proc sort;
by group;*/
proc corr;
/*by group;*/
run;
```

APPENDIX 11

LIPID EXTRACTION AND FATTY ACID METHYLATION PROTOCOL

Sample preparation:

1. **Intact muscle form—**Fillets (excluding belly flap) are skinned, vacuum packed and kept at –20°C. When lipid extraction is performed, frozen fillets are partially thawed and processed the same as powdered sample preparation.

2. **Powder form**—Fillets are skinned, cut into small pieces (excluding belly flap), frozen with liquid N_2, and powdered in a Waring blender for 1-2 min. TBHQ (0.1 g) is added before blending to prevent lipid oxidation. Powdered samples are kept at –80°C.

Important:

- Use 35-mL Teflon-lined screw cap glass centrifuge tubes
- Check the condition of tubes and caps for methylation step—No chips or cracks
- Work under hood
- Use glass when working with chloroform or methanol

Instruments:

1. Hood
2. 60°C water bath or heating block
3. 90°C water bath or heating block
4. Manifold and nitrogen gas
5. Centrifuge
6. Vortex

Chemicals:

1. Trizma/EDTA buffer:
   50 mM Trizma HCl: 7.880 g per 1000 mL dd water
   1 mM EDTA-disodium salt: 0.372 g per 1000 mL dd water
   Mix above stock solutions in a beaker, adjust pH to 7.4 with 5 M or 1 M NaOH, then filter using a 0.2 micron filter storage unit. Buffer is stored at 4°C.
2. C:M:A (chloroform:absolute methanol: gracial acetic acid) 400:200:3 mL
3. 2:1 (chloroform:methanol by volume) e.g. 400:200 mL
4. 4:1 (chloroform:methanol by volume) e.g. 400:100
5. 4% (w/v) H_2SO_4 in anhydrous methanol. Must prepare fresh.
6. Chloroform
7. Anhydrous Na_2SO_4
8. 1-PS Phase separation filters (diameter 9 cm). This filter type takes water out off solution, thus filtered solution is water free.
9. Glass wool
10. Iso-octane. Filter using a 0.45 micron filter storage unit.

Lipid extraction step:

1. Weigh powdered sample in a 35-mL Teflon lined screw cap glass centrifuge tube.
2. Add 5 mL Trizma/EDTA, then vortex for 60 sec.
3. Add C19:0 (Nonadecanoic acid) as an internal standard at 0.3mg/ml.
4. Add 20 mL C:M:A, then vortex vigorously twice (30 sec x 2).
5. Hold tubes at room temperature for 10 min.
6. Centrifuge at 900 x *g* (4000 rpm), 10°C for 10 min.
7. Transfer all lower layer with glass Pasteur pipet over 1-PS filter. Collect filtered sample in a 35-mL Teflon lined screw cap glass centrifuge tube. *(Pre-rinse filter paper 3 times with 5 mL 2:1 C:M each to remove trace silicone residue, and put away filtrate.)*
8. Add 10 mL 4:1 C:M to the upper layer, vortex for 15 sec, and centrifuge at 900 x *g* (4000 rpm), 10°C for 10 min.
9. Again transfer all lower layer over 1-PS filter. Rinse filter paper with 5 mL 2:1 C:M after the filtrate has gone. Take filter paper out, and rinse inside and outside of funnel with 1 pipett of 2:1 C:M. (Filtrated sample can be stored at 0-5°C for 1-3 days.)
10. Blow down under nitrogen gas to dry sample in 60°C water bath. Nitrogen gas outlet should be close to liquid surface. *(It takes around 60-75 mi. Completely dry sample does not have a smell of acetic acid.)*

Methylation step:

1. Add 4 mL of 4% H_2SO_4 solution. *(CAP THIGHTLY-NO LEAKING)*
2. Heat in water bath or heating block at 90°C for 60 min.
3. Cool in to room temperature then add 3 mL dd water (to stop reaction).
4. Add 8 mL chloroform, then vortex for 30 sec.
5. Centrifuge at 900 x *g* (4000 rpm), 10°C for 10 min.
6. Transfer the bottom layer (chloroform layer) through a Na_2SO_4 filled glass Pasteur pipet into 10-mL glass tube. Collect filtered sample in a 10-mL screw cap glass tube. *(Fill Pasteur pipet with glasswool first, then with around 1 inch of Na_2SO_4 layer. Pre-rinse with 1 pipett of chloroform and put away filtrate)*. Rinse the filter layer with a half pipett of chloroform to wash out sample that trapped in the filter layer. Before take the filter out, rinse the outside with a little bit of chloroform.
7. Blow down under nitrogen gas in 60°C water bath. Nitrogen gas outlet should be close to liquid surface. *(It takes around 20-25 min. Completely dry sample does not have a smell of chloroform.)*
8. Resuspend dried sample in 3 mL of filtered isooctane and keep at –20°C or inject into GC.

Calculations:

$RF = (A_x \times C_{is})/(A_{is} \times C_x)$

A_x = area of fatty acid peak
C_x = concentration of fatty acid
A_{is} = area of internal standard
C_{is} = concentration of internal standard

* The **RF** value is then used as a correction factor for calculating concentrations of each fatty acid.

APPENDIX 12

GENOME LAB GeXP-MULTIPLEX GENETIC ANALYSIS SYSTEM PROTOCOL

PRIMER DESIGN:
Make a list of 30-40 genes of interest and obtain accession numbers and sequences.
Insert genes as outlined in GeXP Chemistry Protocol.
Design Multiplex as outlined in GeXP Chemistry Protocol. This will generate a list of primers.
BLAST primers against NCBI Database. Ensure that the primers identify the desired gene and that the reverse primer does not bind a gene with the 5' end (bp 20) with the forward primer binding the same gene. If the forward and reverse primers bind the same gene, the primer set must be redesigned. If either primer binds another gene of interest included in the multiplex, then the primer set must be redesigned. Once the primers have been determined to be desirable then the primers with the universal tag can be ordered.

PRIMER OPTIZIMATION:
Resuspend primers to 100µM concentrations in 10mM Tris-HCl pH8.0

Reverse Primers:
Make reverse plex by making a 1mL mixture of all reverse primers at a 500nM concentration. With 41 primers, take 5µL of each 100µM reverse primer and add 795µL of 10mM Tris-HCl to make the final volume 1mL.

Forward Primers:
Dilute 100µM forward primers to 200nM individual solutions. Take 2µL of 100µM primer and add 498µL of Tris-HCl.

DNase Treatment:
Take 2µg of RNA pool in 5 replicates. Add 2µL of DNase and 2µL of 10x Buffer then make the total volume of the reaction equal 20µL by adding water. The thermocycler should be set to run at 37^{o}C for 1 hr. To stop the reaction, add 2µL of STOP and run on the thermocycler at 60^{o}C for 10 min.

Kan(r) RNA Dilution:
Dilute Kan to 1:50 with RNase/DNase free water (10µL in 490µL of water).

Reverse Transcription Reaction:
Dilute 20µL of DNase treated RNA pool in 80µL of RNase/DNase free water for a total volume of 100µL. Make master mix as outlined below. The total reaction volume should be 10µL. The thermocycler program is under the GeXP folder and is entitled "RT*". The reaction is 48^{o}C for 1min, 42^{o}C for 60min, 95^{o}C for 5min, and 4^{o}C hold.

MasterMix:

H_2O	1.5µL
5x RT	2.0µL
RT	0.5µL
Kan	2.5µL
RevPlex	7.0µL
RNA	0.5µL (H_2O for NTC)

PCR Reaction:
Make a master mix as outlined below. Then add 4.35µL of the master mix, 3.15µL of RT reaction products, and 2.5µL of primers. You can run singlet reactions or reactions with multiple primers. I ran 5 forward primers in each well, so I added 0.5µL of each 200nM forward primer for a total reaction volume of 10µL. The thermocycler program is under GeXP folder and entitled "PCR*".

Master Mix:

$MgCl_2$	2.0µL
5x Buffer	2.0µL
Polymerase	0.35µL

Multiplex (Singlet Reactions):
Dilute PCR products in strip tubes with 2µL of PCR products in 8µL of 10mM Tris-HCl.
In a 96-well plate add:

Diluted PCR products	1.0µL
DNA Size Standard (400bp)	0.5µL
Sample Loading Solution	38.5µL
	40µL total

Add 1 drop of mineral oil to the top of each well
Fill appropriate wells on Buffer Microplate 2/3 full with Separation Buffer
Ready for multiplex
(The plate should be stored in the refrigerator until the machine is ready; the DNA standard is light sensitive.)
Follow standard protocol for setting up the GeXP.

Multiplex (Whole):
Used PCR products of Reverse Plex + full Forward Plex

Reverse Plex— made a 1mL mixture of all reverse primers at a 500nM concentration. With 41 primers, take 5µL of each 100µM reverse primer and add 795µL of 10mM Tris-HCl to make the final volume 1mL.

Forward Plex—combined 5µL of each 100µM forward primer (used 2µL in PCR reaction)

Dilute PCR products in strip tubes with 2µL of PCR products in 8µL of 10mM Tris-HCl.
In a 96-well plate add:

Diluted PCR products	1.0µL
DNA Size Standard (400bp)	0.5µL
Sample Loading Solution	38.5µL
	40µL total

Add 1 drop of mineral oil to the top of each well
Fill appropriate wells on Buffer Microplate 2/3 full with Separation Buffer
Ready for multiplex
(The plate should be stored in the refrigerator until the machine is ready; the DNA standard is light sensitive.)
Follow standard protocol for setting up the GeXP.

RUNNING AN OPTIMIZED MULTIPLEX-PCR REACTION

Reverse Primers:
Combine all 100µM forward primers at 1.25µL except for β-actin (0.35 µL), FAS (0.625 µL), RibProt (0.125µL), and EF1a (0.125µL).

Forward Primers:
Make a 0.5 mL mixture of all forward primers at a 500nM concentration. With 41 primers, take 1µL of each 100µM reverse primer and add 460µL of 10mM Tris-HCl to make the final volume 500µL.

DNase Treatment:
Take 2µg of RNA and add 2µL of DNase and 2µL of 10x Buffer then make the total volume of the reaction equal 20µL by adding water. The thermocycler should be set to run at 37°C for 1 hr. To stop the reaction, add 2µL of STOP and run on the thermocycler at 60°C for 10 min.

Reverse Transcription Reaction:
Use undiluted DNase-Treated RNA. Make master mix as outlined below. The total reaction volume should be 10µL. The thermocycler program is under the GeXP folder and is entitled "RT*". The reaction is 48°C for 1min, 42°C for 60min, 95°C for 5min, and 4°C hold.

MasterMix:	
H_2O	2.75µL
5x RT	2.0µL
RT	0.5µL
Kan [stock]	1.25µL
RevPlex	1.0µL
RNA	2.5µL (H_2O for NTC)

PCR Reaction:
Make a master mix as outlined below. Then add 5.35µL of the master mix and 4.65µL of RT reaction products. The thermocycler program is under GeXP folder and entitled "PCR*". The reaction is 95°C for 10min, 95°C for 30 sec, 55°C for 30sec, 70°C for 1min, repeat steps 2-4 for an additional 34 cycles (35 cycles total), and 4°C hold.

Master Mix:	
$MgCl_2$	2.0µL
5x Buffer	2.0µL
Polymerase	0.35µL
ForPlex	1.0µL
cDNA	4.65µL

Multiplex:
Use undiluted PCR products.
In a 96-well plate add:

PCR products	1.0µL
DNA Size Standard (400bp)	0.5µL
Sample Loading Solution	38.5µL
	40µL total

Add 1 drop of mineral oil to the top of each well
Fill appropriate wells on Buffer Microplate 2/3 full with Separation Buffer
Ready for multiplex
(The plate should be stored in the refrigerator until the machine is ready; the DNA Standard is light sensitive.)
Follow standard protocol for setting up the GeXP.

STANDARD PROTOCOL FOR OPERATING GENOME LAB GeXP GENETIC ANALYSIS SYSTEM:

1. Operation of the GenomeLab GeXP Genetic Analysis System
    - Power up the computer and analyzer. If you hear a long buzz, lift lid, replace and restart.
    - Warm the capillary array and gel to room temperature for ~30 minutes.
    - Open the GenomeLab GeXp software.
    - Access personal database. Set as working database. Create a new project.
    - Select the Run Control Tab. A message will appear indicating a gel error. Hit OK.
    - Select the direct control tab.
    - Right click on the gel cartridge icon and select "Install gel cartridge." Enter the lot number and hours. Load gel cartridge and select "Done."
    - Right click on the capillary array icon and select "remove manifold plug" and then "install capillary array." Enter info and select done.
    - Replace all doors and close cover
    - Right click under direct control and perform **manifold purge**. Change to 3Xs with 0.4 ml gel.
    - Select **Fill Gel Capillary** 3xs.
    - **Perform an optical alignment**→ instrument data options→ scan check.
      Monitor Baseline: Under Run→monitor baseline→select enable→auto save
      To look at channels, Open data monitor. Click the Y axis and select 8K so that all are on the same scale.
      Channel C always runs higher than the others. Do not want above 5K.
      Under Run→monitor baseline→select disable→auto save.
    - Go to Main Menu and select Setup. Enter unknowns into plate map. At the base of the column, select the method (Frag 3) from the pull-down options. Save As
    - Select Run sample plates. **Always remember to change project from default to project of interest**
    - Select load the plate:
        - Load the plate-make sure it is properly aligned. Left side-rear.
        - Clean the water tray-replace with DIwater.
        - Add Separation buffer to corresponding wells of a 96 well buffer microplate (2/3 full) Replace cover.
    - Close the machine cover and start-plated loaded-→load→ OK.

2. After Completion of the Run:
    - Remove gel cartridge: Run→direct control right click on icon→remove gel cartridge. Remove the gel cartridge and return to 4°C. Insert plug.
    - If the machine will not be used within a few days, remove the capillary array.
      Run→direct control right click on icon→release capillary array
      Open both doors and remove covers. Pull out the array with left hand while releasing the manifold plug with the right. Place carefully on bench top. Fill caps with nano water and place on capillary. Ensure the tips are in the water. Place cap on manifold plug. Place protective piece over window of manifold plug. Document usage on the outside of the box. Return to 4°C.

3. Fragment Analysis
    - Main menu→ Fragments→Raw Data→Select project
    - From List View, highlight to select your samples. Use the right arrow to move the selected samples into the Raw Data Field hit Next.
    - Select "GeXP analysis" as parameter set and hit "next" and "analyze". Wait for analysis to complete and ensure that each sample analysis indicates "Pass." If not, this indicates a problem. Select "Finish."
    - A new window will open. You will be prompted to Add Study? Select YES and save using a new study name.
    - Double click "Fragments List" under the Data Tab. Deselect dyes 1-3. Select and apply "mTOR/PPAR/FA Filter Set" under Exclusion filter. Check box indicating "Show Excluded."
    - Data is now ready to be reviewed. Verify that all expected peaks are present and that all peaks are identified as a single peak. One peak area that is critical is ~165 nt. We want to select for the second of the double peak even if the area is smaller. This can be accomplished by tweaking the filter exclusions.
    - Export data for further analysis by selecting "transport fragments for GeXP" under the File pull-down menu. Save to K drive.

4. Analysis using Profiler Software
    - Open Profiler Software using "bcleveland" log-in info.
    - Select "Express Analysis". Log-in info is required at this step as well.
    - Under GeXP analysis tab, Create a new analysis. Give a unique name to the analysis. Analysis set up window will open. Select FA_15 from multiplex list. Save
    - Select GeXP import to pull in data from K drive using "Browse." Add plate to the database. Save.
    - Under Plate Set up Tab, open plate and open sample layout. Highlight wells and assign multiplex. Save
    - Open GeXP analysis normalization. Normalize to Kan peak. Select ALL under display normalized values.
    - Look over results to fine-tune. Adjust peak binning to capture/eliminate peaks as needed. Save.
    - Open report View. Select report format: Profile by gene. Hit export data. **Must assign .TXT to the file name.** Save to K drive.

5. Further analysis in Excel
    - Further analysis includes utilizing the standard curve generated for each gene and normalization prior to being further processed in GeNorm.

VITAE

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Effects of maturation, polyploidy, and nutrition on growth, composition, and gene expression within fatty acid metabolism in rainbow trout

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